STUDIES IN INFLUENZA AND PNEUMONIA

VII. A STUDY OF THE EFFECTS FOLLOWING THE INJECTION OF BACTERIA FOUND IN INFLUENZA IN NORMAL THROATS, IN SIMPLE NASOPHARYNGITIS, AND IN LOBAR PNEUMONIA

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INTRODUCTION

It is my purpose to record in this paper the results obtained from the injection in various ways into animals of material obtained from patients with influenza and influenzal pneumonia and from sources other than influenza, to give the important facts in a series of cases of influenza in which the findings in the patients and the results from the injections of animals are correlated, to describe and illustrate the gross and microscopic changes that followed the injection of the bacteria

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from influenza, and to compare these changes with those noted in influenzal infection as it occurred during the epidemic of 1918 to 1919.

In a previous report [21, 22] it was pointed out that the streptococci from patients with influenza when injected intraperitoneally into mice and guinea-pigs possessed high virulency, that following these injections, lesions of the lungs and pleura occurred frequently, and that the animals often showed respiratory embarrassment during life and voluminous, emphysematous, and sometimes hemorrhagic lungs after death (case 2607, guinea-pig 737; table 2).

These findings in the experiments with streptococci suggested that the direct application of influenzal material to the normal, uninjured mucous membrane of the trachea and bronchi of animals might result in the production of lesions more marked than those following intraperitoneal injection, and should this be true, it might be possible to throw light on the mechanism of infection in this disease, and to compare the lesions obtained under controlled conditions of dosage and type of micro-organism in animals with those in man. The experiments on intraperitoneal injection and many clinical findings such as the relatively immobile, expanded thorax, the wheezing râles, the dyspnea, cyanosis, and leukopenia suggest strongly that influenza may be in part an anaphylactic reaction. The voluminous lung noted so commonly after death is another argument for this view. The guinea-pig, known to respond more like man than any other animal with respect to anaphylactic reactions, was selected as probably the most suitable in which to study the pulmonary and other lesions following the injection of influenzal material.

TECHNIC OF INTRATRACHEAL INJECTION

The technic of intratracheal injection should be such as to make it quite impossible to injure materially the lining of the trachea and bronchi. Discarded ureteral catheters cut at an angle of 45 degrees with margins rounded have been found to fulfill this requirement. The guinea-pig is wrapped in a towel; the head is held in place by the handles of an inverted artery forceps. The mouth is held open by spring wire retractors, and the tongue is depressed by a suitable small instrument. Under a strong reflected light, properly shaded, the catheter is inserted into the larynx with a quick stroke before the contraction of the muscles of the epiglottis can divert the tube into the esophagus. The animal's sharp, quick cough and total inability to use its voice, and the sensation of the catheter's passing the tracheal rings, indicate that it has entered the trachea. The catheters are sterilized by boiling and in order to avoid the possibility of transmitting accidental infection from one guinea-pig to another, a separate, freshly sterilized catheter was used for each animal in this series. Care was exercised to use only healthy, vigorous, and active animals from stock that was free from epidemic disease. At first the dose of

culture given was very small, and the results in consequence were too irregular to permit accurate analysis. Later the dose was increased; 0.1 c c of the sputum or exudate and 0.5 c c of the glucose-blood or brain-broth culture for each 100 gm. of body weight were used in the experiments reported unless it is otherwise indicated. The cultures for injection were incubated at from 33-35 C. for from eighteen to twenty-four hours in tall columns of glucose-brain broth or glucose-blood broth. Control cultures of the material injected were always made on blood-agar plates. This was found necessary not only in order to prove the viability of the organisms injected, but also in order to determine the type that had grown in the particular culture. As has been pointed out heretofore,[20] the most important organism found in the sputum in influenza was a gram-positive, often lanceolate diplostreptococcus which produces greenish colonies on blood-agar plates. The colonies are larger, flatter, and more moist than those of Streptococcus viridans, often indistinguishable from pneumococcus colonies. In this report I shall designate this organism, including the strains that ferment inulin, as a "green-producing streptococcus" or "green streptococcus" to distinguish it from Streptococcus viridans.

Control experiments were first made to determine the harmlessness of intratracheal injection of varying amounts of salt solution and sterile broth. All guinea-pigs injected with salt solution (1 with 6 c c; 3 with 3 c c, and 1 with 2.5 c c) remained well. They showed a slight increase in respiration immediately after injection. All were free from symptoms the following day and remained so. Fifteen guinea-pigs were injected with glucose broth, glucose-blood broth, or glucose-brain broth (4 with 3 c c; 3 with 2.5 c c, 7 with 1.5 c c, and 1 with 1 c c). They showed relatively slight respiratory disturbance immediately after injection. Some, especially those injected with meat infusion-peptone-glucose-blood broth, showed mild symptoms of anaphylaxis. They coughed, scratched the nose with their paws; and were irritable for a short time after injection. All were well the day after injection, and all but two remained well subsequently. The one which had been injected with 1.5 c c died thirteen days later from an old bronchopneumonia that showed Bacillus bronchisepticus. The other, which had been injected intratracheally with 3 c c glucose broth, died ten days later with bronchopneumonia and a moderate amount of bloody fluid in the pleural cavities. Cultures from the blood showed a few colonies of green-producing streptococci, and from the pleural fluid, staphylococci and B. bronchisepticus. The culture in glucose-brain broth of the green-producing streptococci from the blood was injected intratracheally in 3 guinea-pigs. Two had slightly increased respirations for two days and then recovered. The other had no symptoms; it was chloroformed three days after injection and showed no lesions. None of the guinea-pigs showed leukopenia. The average leukocyte count before injection was 9,600, twenty-four hours after injection 9,100, and forty-eight hours after injection, 12,200.

INCIDENCE OF OCCURRENCE OF VOLUMINOUS LUNGS, HEMORRHAGIC EDEMA OF BRONCHOPNEUMONIA, AND PLEURITIS FOLLOWING INTRAPERITONEAL AND INTRATRACHEAL INJECTION OF SPUTUM, LUNG AND OTHER EXUDATES, AND CULTURES FROM PATIENTS WITH INFLUENZA IN RELATION TO MORTALITY

The more marked effects of intratracheal injection over intraperitoneal injection of material from patients with influenza became apparent at once. The symptoms of respiratory embarrassment were more pronounced and the lungs more voluminous. In table 1 the

average volume and weight of the lung of a series of guinea-pigs injected intratracheally, and of normal guinea-pigs are given (fig. 1). The volume of the lungs in cubic centimeters, as measured by displacement of water for normal guinea-pigs weighing about 350 gm., and killed with chloroform, was 6.5 c c, or approximately one-fiftieth or 2% of the weight of the animals expressed in grams. The average volume of the lungs of guinea-pigs that had died from causes other than pneumonia was found to be about normal. The average weight of the lungs was found to be 3.3 gm., or about 1% of the body weight. It is evident from table 1 that the more toxic or virulent the culture, the more severe the reaction in the lung, and the earlier the death occurred following intratracheal injection, the greater was the volume and weight of the lung. Thus the volume and weight were approximately three and four times the normal in the guinea-pigs dying in two and one-half hours and two days after injection, respectively, and in those that died in three days the average volume of the lung was less than twice that and the weight about two and one-half times that of the average normal.

TABLE 1

RESULTS OF INTRATRACHEAL INSUFFLATION OF CULTURES FROM INFLUENZA AS SHOWN BY VOLUME AND WEIGHT OF LUNGS

	Average Weight of Animals, Gm.	Average Volume of Lungs, C C	Average Weight of Lungs, Gm.
Guinea-pigs living an average* of 2.5 hours.....	410	19	13
Guinea-pigs living an average of 2 days.....	390	17	14
Guinea-pigs living an average of 3 days.....	340	10	8
Normal guinea-pigs (controls).....	350	6.5	3.3

* The averages of 6 guinea-pigs in each series are given.

The increase in lung volume was about the same following the injection of sputum, primary culture of sputum, pure cultures of freshly isolated strains of green-producing streptococci, hemolytic streptococci and staphylococci (usually in the second or third generation). The average volume of the lung of a large number of guinea-pigs after intratracheal injection of influenzal material was 15 c c, and after intraperitoneal injection 10 c c; after injection of type pneumococci it was 10 c c and 7 c c, respectively.

From table 2 may be obtained a general picture of the differences in the results obtained in the mortality and incidence of lesions of the lungs in guinea-pigs injected intratracheally and intraperitoneally with material from patients with influenza, with cultures from normal

throats during the epidemic and after it had subsided, with type pneumococci, and with sputum and cultures from patients with simple nasopharyngitis and tracheitis. The total average mortality following injection of material from 111 cases of influenza in 192 animals was

TABLE 2

MORTALITY AND INCIDENCE OF LESIONS OF THE LUNGS IN GUINEA-PIGS INJECTED WITH MATERIAL FROM INFLUENZA; WITH CULTURES FROM NORMAL THROATS DURING AND AFTER THE EPIDEMIC; WITH TYPE PNEUMOCOCCI AND WITH SPUTUM AND CULTURES FROM PATIENTS WITH SIMPLE NASOPHARYNGITIS AND TRACHEITIS

Material Injected	Place of Injection	Number of Strains	Number of Animals			Percentage of Mortality	Percentage Showing		Pleuritis
			Injected	Recovered	Died		Voluminous Lungs	Hemorrhagic Edema or Pneumonia	
Sputum	Trachea	16	17	5	12	70	64	62	54
	Peritoneum	38	48	15	33	68	25	8	14
Primary culture	Trachea	17	31	16	15	48	36	55	15
	Peritoneum	12	13	7	6	46	22	22	11
Green-producing streptococci	Trachea	19	33	17	16	48	73	54	26
	Peritoneum	12	16	7	9	56	58	17	42
Hemolytic streptococci	Trachea	9	17	7	10	59	83	71	44
	Peritoneum	4	6	2	4	67	67	0	0
Staphylococci	Trachea	6	11	5	6	55	71	43	43
Total for influenza (111 cases)		133	192	81	111	58	55	46	28
Primary culture of throats of normal persons during epidemic of influenza	Trachea	4	12	7	5	42	17	17	0
Primary culture of throats of normal persons months after epidemic had disappeared	Trachea	15	15	12	3	20	0	20	0
Type I, II, III, and IV pneumococci from lobar pneumonia	Trachea	14	20	14	6	30	30	35*	35
	Peritoneum	14	18	0	18	100	6	0	28
Sputum and cultures from patients with simple nasopharyngitis and tracheitis	Trachea	2	10	8	2	20	0	20	0

* Lobar pneumonia.

58%. The total average incidence of voluminous lungs was 55%; of hemorrhagic edema or bronchopneumonia, 46%, and of pleuritis, 28%. The killing power of the influenza strains was only slightly lower when they were applied to the normal mucous membrane of the trachea and bronchi than when they were injected intraperitoneally. The results

of control experiments with type pneumococci were quite different in this respect; their killing power was 100% on intraperitoneal injection, whereas only 30% of the animals died following intratracheal injections of the same dose. The average incidence and the degree of lesions of the lung and pleura were higher, as would be expected, following intratracheal injection than following intraperitoneal injection.

The property in these strains which caused symptoms resembling anaphylaxis, voluminous lungs with acute hemorrhagic edema of lungs and leukopenia, and the general virulency, tended to disappear promptly on artificial cultivation, especially if the organisms were cultivated under aerobic conditions. To illustrate:

The volume of the lungs in 2 guinea-pigs was 20 and 12 c c, respectively (average 16 c c), following injection of the primary culture from the blood of a patient with influenza containing a pure culture of the green-producing streptococcus while that in 2 guinea-pigs injected with the same strain in glucose broth after one plating on blood agar was 12 and 10 c c (average 11 c c), and the hemorrhage and edema of the lung were much less marked. Different strains differed markedly in the loss of this power, depending to some extent on the method of cultivation. Aerobic cultivation on blood agar destroyed these peculiar properties rapidly, while by rapid transfers of glucose-brain broth from tube to tube they might be retained for many generations. The typical picture has followed injection of strains in the eleventh culture generation. The tendency of some of the strains to localize and produce a certain type of lesion was striking, often corresponding to the type of lesions found in the patient. Thus, in a case of death from hemorrhagic edema of the lung with pseudolobar pneumonia and hemorrhagic pleuritis (case 2800), the cultures from the throat in the first and second culture generation produced hemorrhagic edema of the lung and hemorrhagic pleuritis in two guinea-pigs injected into the trachea (g. pigs 947 and 956), and in one injected into the stomach (g. pig 948). The same strain injected about three months later when in the sixth subculture had lost much of its virulency, but it still localized in the same manner and produced bronchopneumonia with localized abscesses in the lung and adhesive pleuritis resulting in perforative peritonitis (g. pig 1311).

Experiments with Lung and Other Exudates.—During the course of the experiments the effects of injecting directly the lung exudates of patients and of lung emulsions, peritoneal, and pleural exudates from animals was also studied. Contrary to expectations, the symp-

toms and lesions following direct injection were less acute than those following injection of the cultures made from these exudates and following the injection of sputum and cultures of streptococci from the sputum and throat. The mortality following direct intratracheal injection of the exudates from 16 cases into 19 guinea-pigs was only 42% as compared, for example, with a mortality of 70% following injection of sputum. The mortality in the 19 guinea-pigs was almost wholly due to injection of peritoneal and lung exudates in guinea-pigs dead from injection of sputum or cultures. Most of the animals that died showed bronchopneumonia, and only a few acute hemorrhagic edema. This relatively low mortality was not due to a lesser number of viable organisms injected because plate cultures often showed a larger number of living bacteria than were present in the sputum or cultures. Under the conditions of a more forced experiment, intraperitoneal injection, the mortality in 11 guinea-pigs injected with 11 strains was higher (64%). Moreover, the theory that these bacteria when soaked in blood or lung exudate tend to lose their bite, as it were, when applied to the normal pulmonary epithelium is further borne out by the fact that the virulency and incidence of acute hemorrhagic edema were higher following injection of cultures from the sputum and throat than of cultures from the blood. Thus, in one case (case 2,800) the mortality following intratracheal injection of the throat and sputum strains was 64%, while following injection of the strain isolated from the blood it was 33%.

Intratracheal Injections of Influenza Bacilli. — Recently isolated strains from the throats of 5 undoubted cases of influenza — 4 in the second, and 1 in the sixth culture generation — were injected intratracheally into 5 guinea-pigs. The dose was 0.5 c c for each 100 gm. body weight of a dense salt suspension from rich growths on chocolate blood agar. The amount of culture injected ranged from the growth of from 1-5 slants. In 3, leukocyte counts were made; 1 of these showed a drop from 12,000 before injection to 6,800 twenty-four hours after injection and 8,000 seventy-two hours after injection. The others showed no change in the leukocyte count. All the animals recovered. Besides slightly increased respiration immediately after injection there was no noticeable effect, and all the animals seemed quite well without increased respiration or rise in temperature 24 hours after injection. The virulency of 2 of these strains was proved in a mouse. Injection of 0.4 c c of a mixture of 3 of the strains killed the mouse in 24 hours. The animal showed enormous subcutaneous

hemorrhages in the right groin adjacent to the point of the intraperitoneal injection, and there were hyperemia of lungs and a number of subpleural hemorrhages, but no gross evidence of peritonitis. The cultures from blood and peritoneal fluid on chocolate and blood-agar plates yielded countless numbers of influenza bacilli. In connection with these experiments with pure cultures of influenza bacilli should be considered the fact that intraperitoneal injections of sputum into mice and intraperitoneal and intratracheal injections into guinea-pigs were never followed by invasion by influenza bacilli as determined by cultures, direct examination of smears, and microscopic examination of

TABLE 3

PREDOMINATING ORGANISM IN SPUTUM AND PRIMARY CULTURES FROM SPUTUM INJECTED IN ANIMALS AND FOUND IN THE ANIMALS THAT DIED

Material Injected	Place of Injection	Incidence of Predominating Organism in							
			Material Injected				Animals That Died		
		Strains	Green-producing Streptococci, per Cent.	Hemolytic Streptococci, per Cent.	Staphylococci, per Cent.	Animals Cultured	Green-producing Streptococci, per Cent.	Hemolytic Streptococci, per Cent.	Staphylococci, per Cent.
Sputum	Trachea or peritoneum of guinea-pigs	54	71	20	9	29	81	4	15
Primary culture from sputum	Trachea or peritoneum of guinea-pigs	29	59	24	17	22	73	14	13
Sputum	Peritoneum of mice	19	69	21	10	17	82	12	6
Total.....	.....	102	68	20	12	68	78	9	13

sections stained for influenza bacilli as recommended by MacCallum.[17] In some of the sputums thus injected large numbers of influenza bacilli were demonstrated in smears and by cultures before injection. Thus in the lung of the guinea-pig shown in figures 5, 10 and 11 the sputum injected contained large numbers of influenza bacilli, but they were absent in the peritoneal exudate, blood and lung tissue. The invasive power of freshly isolated influenza bacilli (virulent to mice on intraperitoneal injection) and of those in the sputum itself when applied to the tracheal mucous membrane in guinea-pigs was found to be slight as compared with the invasive power of the streptococci. It is possible that tracheal injection of adapted strains or those whose virulency is

enhanced by animal passage through intraperitoneal injection might acquire the power to invade the lung and produce bronchopneumonia and possibly hemorrhagic edema of the lungs.

The Comparative Invasive Power of the Bacteria from Patients with Influenza. — The high invasive power of the green-producing streptococcus became apparent early in the work. We have determined which of the different bacteria occurred in predominating number in the sputums injected into guinea-pigs and mice and in the primary cultures from sputum injected into guinea-pigs, and also in exudates and blood of the animals that died as a result of the injections. In table 3 is given the percentage of incidence of the predominating organisms. It will be noted that the green-producing streptococcus was the predominating organism in the material injected in each group of experiments and, what is more significant, it was the predominating organism in a higher percentage of the animals after death, whereas the reverse was true of hemolytic streptococci and staphylococci. The relative importance of these three organisms in influenza might be said to be indicated roughly by the figures in the last line of table 3.

PROTOCOLS OF EXPERIMENTS FOLLOWING INJECTION OF MATERIAL FROM INFLUENZA

Guinea-pig 846, weighing 420 gm., was injected intraperitoneally Dec. 28, 1918, with 2.5 c c of glucose-broth culture of the green-producing streptococcus from the blood of G. pig 828, which had been injected with the sputum from case 2,749. December 29 the animal was found dead. Marked hemorrhagic serofibrinous peritonitis, moderate distention and congestion of the lungs (11 c c), a large number of large and small subpleural hemorrhages, and beginning pleuritis were found. The pleura contained 3 c c of turbid, blood-tinged, chocolate-colored fluid. A moderate amount of bloody, frothy fluid escaped from the cut surface of the lungs. The uterus contained several hemorrhagic areas marking placental attachment. The cultures from the blood, pleural fluid, peritoneal fluid, and placental site showed many green-producing streptococci.

Guinea-pig 981, weighing 470 gm., was injected Jan. 16, 1919, intratracheally with 1.5 c c of the glucose-broth culture of the green-producing streptococcus in the second culture generation isolated from the blood in case 2,800. The white blood count before injection was 8,000; the temperature 102.2 F. January 17 the animal appeared less active than normal and the respirations were slightly increased. The leukocyte count was 2,000 and the temperature 103.2 January 18 the animal was more active, but the respirations were still slightly increased. The leukocyte count was 6,200, the temperature 102.8. January 19 the animal appeared quite well. The respirations were normal, the leukocyte count was 12,200, and the temperature 102.4. The animal made a complete recovery; when it was chloroformed January 24 it showed no lesions. The cultures from the blood and lung remained sterile.

Guinea-pig 995, weighing 470 gm., had injected into the trachea, Jan. 18, 1919, 1.5 c c of the glucose-broth culture of the green-producing streptococcus in

the second culture generation isolated from the blood of case 2,800. The white blood count before injection was 9,200, the temperature 102.4. There were moderate symptoms of dyspnea immediately following injection. January 19 the animal appeared quite well but the respirations were definitely increased. The leukocyte count was 6,200, the temperature 103. January 20 the respirations were slightly increased, the leukocyte count was 14,400, the temperature 103. January 25 the animal appeared well. February 24 at 7:30 a. m. the animal appeared ill. The respirations were markedly increased and difficult. The animal when taken from the cage and placed on a table had a typical attack resembling anaphylactic shock with bronchial spasm. At noon the respirations were exceedingly rapid and the animal appeared to be very sick; at 4 p. m. it was found dead. The white blood count was 12,800. There was a large amount of bloody, turbid fluid in both pleural cavities; the right contained a moderate amount of adherent, partially organizing fibrin. The lungs were collapsed and the intermediate lobe was completely consolidated, grayish-red, and covered with a film of fibrin. There were several thickened areas in the mucous membrane of the uterus indicating a resorption of fetuses. The ovaries were normal. The heart muscle was grayish-red. Cultures from the blood and pleura showed green-producing streptococci.

Guinea-pig 1335, weighing 420 gm., was injected intratracheally, May 15, 1919, at 9:50 a. m. with 1.5 c c of glucose-acacia-broth culture of staphylococcus in the sixth subculture from case 2,623. At 10:15 a. m. respirations were rapid, difficult and irregular. There were repeated attacks bordering on bronchial spasm, expiration was forced and prolonged, and the animal was weak. At 10:30 a. m. the respirations were extremely difficult. The animal coughed violently at intervals making desperate efforts with each expiration, and during one of these violent efforts it ran about aimlessly with blood spurting from its nose and mouth; it fell on its side and died with its head in a pool of frothy blood. The lung was found greatly distended (20 c c) and hemorrhagic and edematous throughout. Sections showed marked distention of alveoli and destruction of the epithelium lining the alveoli and of the endothelium of the capillaries. In areas dissolution was so marked as to make it quite impossible to distinguish the alveolar boundaries.

Guinea-pig 956, weighing 280 gm., was injected intratracheally, Jan. 13, 1919, with 1.5 c c of glucose-brain-broth culture of the green-producing streptococcus from a single colony on a blood-agar plate inoculated with the swab from case 2,800. January 14 the animal was very ill; respirations were rapid and difficult. The fur was rough and the animal was restless and irritable. It died at noon. The lungs were markedly distended (15 c c). Both diaphragmatic lobes were dark and mottled. The cut surfaces everywhere were extremely moist and a large amount of bloody, frothy fluid exuded. The other lobes showed smaller areas of hemorrhagic edema. The alveoli were extremely distended, in places almost to the point of rupturing. The peribronchial glands were edematous. The trachea and bronchi were extremely hyperemic and contained a large amount of bloody, frothy fluid. There was a moderate amount of slightly turbid, blood-tinged fluid in the pleural and pericardial sacs. The mucous membrane of the nose was hyperemic. The suprarenals were swollen and there was cloudy swelling of the kidneys. The mucous membrane of the uterus was markedly hyperemic throughout and showed three hemorrhagic areas marking placental attachments. The vagina contained a moderate amount of bloody mucus. Cultures from the blood showed one colony of green-producing streptococci; from the lung and hemorrhagic areas of the mucous membranes of the uterus, large numbers of green-producing streptococci; from

the spleen, kidney, liver, and suprarenals, no growth. In sections of the lung were noted marked dilatation of alveoli, marked desquamation of the epithelial lining of bronchi, and marked edema and hemorrhage in the alveoli with little cellular infiltration (fig. 13 b). The Gram stain showed enormous numbers of streptococci distributed particularly along the alveolar walls (fig. 14 c).

Guinea-pig 947, weighing 400 gm., had injected into the trachea, Jan. 10, 1919, 11 a. m., 1.5 c c glucose-brain-broth culture from the throat swab of case 2,800. At 6 p. m. the respirations were rapid and shallow; the voice was weak and the animal appeared sick. January 13 at 7:30 a. m. the animal was found dead. There was a large amount of hemorrhagic, dark colored fluid in the pleural cavities, containing practically no fibrin. The pleura was rough and covered with a thin fibrinous film, and the lung was compressed by the hemorrhagic fluid in the pleural cavities. The right diaphragmatic and intermediate lobes were extremely wet and edematous on the cut surface. A large amount of bloody, frothy fluid escaped. Portions of the diaphragmatic lobe barely floated in water. The mucous membranes of the bronchi, trachea, and nose were extremely hyperemic and covered with a bloody, frothy fluid. The uterus contained four hemorrhagic areas marking placental attachments. A moderate amount of bloody mucus was found in the uterus and vagina. The stomach was distended with gas rich in carbon dioxid, and showed marked postmortem digestion of the mucous membrane. The cultures from the blood showed a small number of colonies of Staphylococcus aureus and hemolytic streptococci; from the lung, pleura, pericardial fluid, and uterus, large numbers of hemolytic streptococci and staphylococci, and a few from the kidney, liver, and suprarenals. Sections of the lung showed extreme interstitial edema and hemorrhage with marked disintegration of the cells lining the alveoli. The gram stain showed enormous numbers of gram-positive diplococci in the pleura and subpleural spaces in the interstitial tissues and around the blood vessels (fig. 20 a and b).

Guinea-pig 948, weighing 300 gm., was injected intragastrically, Jan. 10, 1919, with 1.5 c c of the glucose-brain-broth culture from the throat of case 2,800. January 13 it appeared to be quite well. January 15 it appeared to be quite well, but was less active and sat humped up. January 19 it sat humped up with ruffled fur, with a dry crust about the nostrils, markedly increased respirations, labored and forced expirations and dilated chest. It had lost 40 gm. in weight. January 20 at 8 a. m. the breathing was rapid and difficult and the animal very much weaker. At noon when it was found dead, it weighed 250 gm. The thorax was distended with a large amount of chocolate-colored fluid (15 c c); there were marked pleuritis with loose fibrinous adhesions throughout and a thin layer covering the pleura. The peribronchial lymph glands were much enlarged and edematous; the lungs were collapsed, but hemorrhagic, and showed areas of bronchopneumonia with white necrotic spots in the intermediate lobe. The pancreas was edematous, the spleen enlarged, and the myocardium gray. No lesions in the stomach or intestinal tract were demonstrable. Cultures from the blood showed green-producing streptococci; those from the pleural fluid, green-producing streptococci and staphylococci.

Guinea-pig 1311, weighing 320 gm., was injected intratracheally, March 25, 1919, with 0.2 c c of the glucose-brain-broth culture from the throat swab from case 2,800 in the sixth subculture. March 26 and 28 the animal's respiration was increased but otherwise it appeared well. April 15 it was found dead and showed three circumscribed areas of necrosis with softening in the left

diaphragmatic lobe. The pleura over each of these areas was bound down by organizing adhesions. One area over the diaphragmatic lobe showed localized hemorrhages and fibrinous deposit on the peritoneal side of the diaphragm, undoubtedly the source of the serofibrinous peritonitis. One area of consolidation in the right cardiac lobe occupying one third of its volume showed recent diffuse consolidation. The white necrotic areas on the cut surface were wedge-shaped and resembled infarcts; the urinary bladder contained a number of circumscribed hemorrhages in the mucosa; but no other lesions were noteworthy. Cultures from the blood, lung, peritoneal and pleural fluids showed green-producing streptococci.

Guinea-pig 1030, weighing 400 gm., was injected intratracheally, Jan. 22, 1919, with 1.5 c c of the lung emulsion from case 2835. The leukocyte count before injection was 10,200; twenty-four hours after injection it was 7,800, and the animal appeared quite well. February 3 the animal was chloroformed. It showed one large grayish-red area of consolidation posteriorly in the left diaphragmatic lobe and one area of consolidation which was dark red and edematous, and a localized necrotic, wedge-shaped, adherent area over the right diaphragmatic lobe.

The experiments cited in detail are representative of a much larger series. Examples of mild effects, mild early effects and severe late effects and marked progressive symptoms from the time of injection are given following injection of pure cultures of the green-producing streptococcus in the first to the sixth subculture, staphylococcus in the sixth subculture, and of a lung emulsion.

CONTROL EXPERIMENTS WITH CULTURES FROM THE THROATS OF NORMAL PERSONS DURING AND AFTER THE EPIDEMIC. THE DETECTION OF THE CARRIER STATE

The occurrence of voluminous lung, hemorrhagic edema, and bronchopneumonia associated with leukopenia following intratracheal injection of the influenza strains was a striking picture. It was thought that intratracheal injection of primary mass cultures from throats of normal persons might detect carriers of the influenza streptococcus. Two sets of experiments were done, one on persons in an institution during the prevalence in the disease in epidemic proportions, the other four months later when influenza had entirely subsided. In the former set, 12 guinea-pigs were injected with cultures from 4 patients. Seven of the guinea-pigs recovered and 5 died, a mortality of 42% (table 2). In 2 cases the animal injections were without apparent effects; in one, the 2 pigs injected died in three and four days, respectively, of bronchopneumonia without leukopenia. The lungs were only slightly enlarged (average volume 10 c c), and quite dry on the cut surface; the exudate was highly cellular. After death green-producing streptococci, or pneumococci, and staphylococci were isolated from the lung in large num-

bers. The streptococci were not agglutinated by the monovalent serum. The results in the fourth case were in sharp contrast. The 3 guinea-pigs and 1 mouse which were injected died. One pig and the mouse were injected subcutaneously, the other animals intratracheally. The 3 guinea-pigs showed a sharp drop in leukocytes. Death in all was due to green-producing streptococci (which were agglutinated by the monovalent serum) and staphylococci. The average leukocyte count before injection was 14,000, while twenty-four hours after injection it was 4,260, a loss of 64%. The two animals injected intratracheally showed voluminous hemorrhagic lungs.

Guinea-pig 1004, weighing 350 gm., was injected intratracheally Jan. 19, 1919, with 1.5 c c of the glucose broth culture from the throat of a normal person who had been exposed to influenza (case 2839). The leukocyte count was 13,000. January 20 the leukocyte count was 4,200, and there was marked shortness of breath and difficult breathing. January 21 at 4 p. m. the animal was found dead, with its head lying in a pool of hemorrhagic fluid. The white blood count was 2,600. The lung was distended, very heavy (14 c c and 12 gm.) and almost completely filled with a frothy hemorrhagic fluid. There was little evidence of consolidation. The peribronchial lymph glands were hemorrhagic and edematous. A thin fibrinous film covered the posterior aspect of the lung. Cultures from the blood, lung, suprarenals, and liver showed staphylococci and green-producing streptococci; those from the kidney and spleen were negative.

The experiments with cultures from normal persons months after the epidemic had subsided consisted of the intratracheal injection of 15 guinea-pigs with cultures from the throats of 15 persons. Of these animals, 12 recovered without developing noteworthy symptoms and 3 (20%) died (table 2). None of these animals showed the violent respiratory embarrassment noted in the animals following injection of the influenza strains. The lung picture after death was quite different than that following injection of the influenza strains. The animals died in one, two and five days, respectively, of green-producing streptococci or pneumococci in the two former, and of Bacillus mucosus in the third. The lungs averaged 8.5 c c in volume. The hemorrhages and consolidation were situated immediately around the bronchi and the consolidated areas were relatively dry on the cut surface, and the exudate highly cellular in character, as noted in the following experiment:

Guinea-pig 1364, weighing 280 gm., was injected intratracheally July 24, 1919, with 1.5 c c of the glucose brain-broth culture from the throat of a normal person (case 3515). July 25 the respirations were decidedly increased. At 4:30 p. m. it was found dead. The thorax was not distended. The pleural cavities were free from fluid; the lungs were only slightly distended (9 c c). There were areas in the diaphragmatic lobes of hemorrhage and beginning infiltration. The cultures from the blood were negative; cultures from the lung showed green-producing streptococci.

CONTROL EXPERIMENTS WITH CULTURES FROM THROATS AND SPUTUM OF PATIENTS WITH SIMPLE NASOPHARYNGITIS AND TRACHEITIS AND WITH CULTURES FROM THE NOSE OF NORMAL GUINEA-PIGS

In some cases there was a marked parallelism between the findings in the patient and in the animals injected (case 2798). Hence it was thought worth while to inject the sputum and cultures from patients with simple nasopharyngitis and tracheitis long after the epidemic had subsided and compare the results with those obtained following injection of the strains from influenza. The results are summarized in table 2. Thus, of 10 animals injected from cultures from 2 cases, 2 died (20%), both of bronchopneumonia. None showed marked respiratory embarrassment resembling anaphylaxis, and none died of hemorrhagic edema. Both of the patients showed leukocytosis, and leukocytosis was the rule in the animals injected.

In order still further to control the results of the experiments on intratracheal injections and to make sure that the bacteria of the upper respiratory tract might not be carried into the lung with the catheter, a series of guinea-pigs were injected with the cultures from freshly isolated strains of the green-producing streptococci normally present in the nose in some guinea-pigs.

The strains isolated from the nose of 2 guinea-pigs were each injected in the usual dose into the trachea of 2 guinea-pigs. The leukocyte count from the guinea-pig in which the culture was made was normal and remained so following injection of sterile broth. The 4 guinea-pigs injected were well the day after injection and remained so for a month thereafter. None of the animals showed leukopenia. All showed a slight rise in leukocyte count the day after injection, and only one a moderate reduction forty-eight hours after injection. The average leukocyte count before injection was 12,550, twenty-four hours

after injection, 14,750, and forty-eight hours after injection, 10,200. It is thus apparent that the chance of carrying infection from the upper respiratory tract through the trachea is extremely slight, not even occurring when large doses of the normal flora of the upper respiratory tract are introduced.

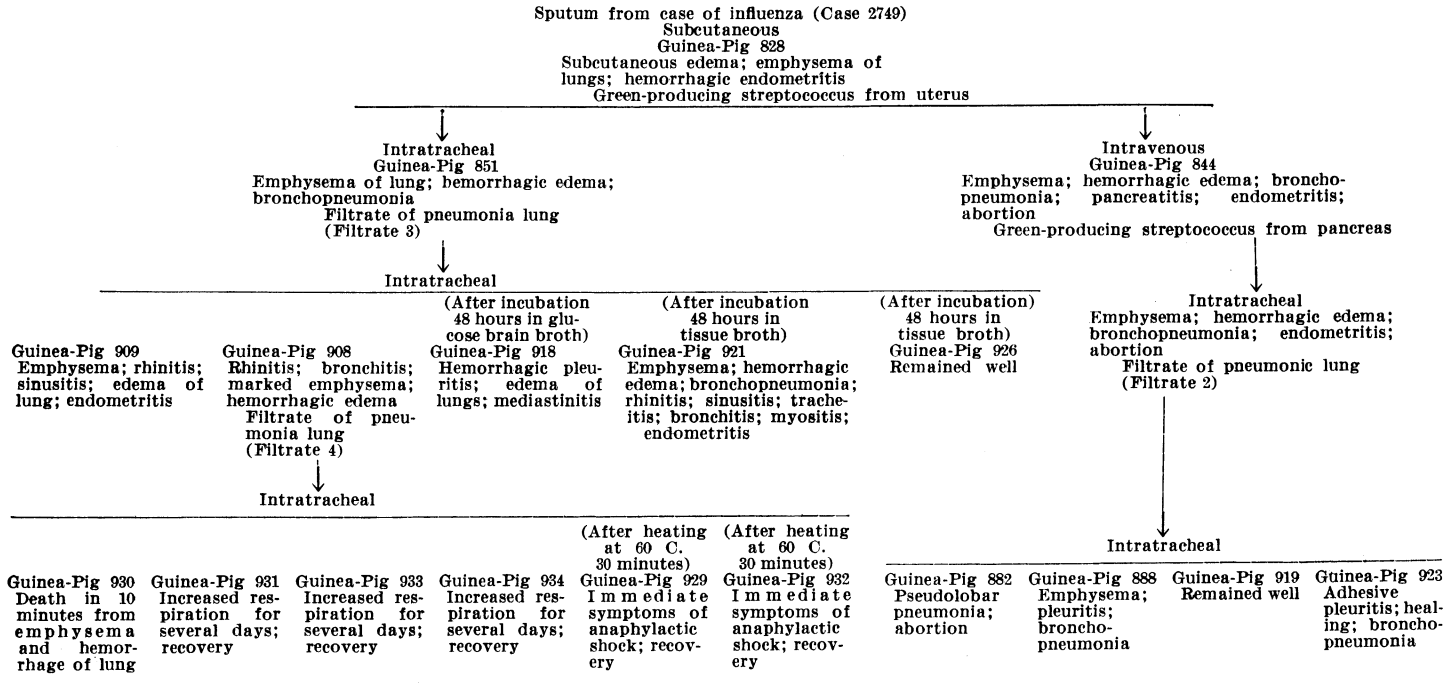
CONTROL EXPERIMENTS WITH TYPE PNEUMOCOCCI FROM LOBAR PNEUMONIA

In order that a correct standard for comparison might be had in evaluating the results of the experiments in influenza, it was considered necessary not only to inject animals with type pneumococci, but also with strains having at least the same killing power, when injected intraperitoneally, dose for dose, as had the strains from influenza. The virulency of the strains were first determined for mice. Twelve strains * of the various types were injected intraperitoneally into 13 mice, the dose ranging from 0.8 to 1.0 c c of glucose-blood or glucose brain-broth culture. All died of peritonitis. The blood and peritoneal fluid in all showed large numbers of pneumococci, the latter often a few colonies of staphylococcus in addition. These 12 strains freshly isolated from the blood, and 2 other recently isolated strains, 14 in all, were then injected intraperitoneally and intratracheally into guinea-pigs. The doses in both were the same and varied from 0.3 to 3 c c of twenty-four-hour glucose brain broth cultures; the usual dose in these, as in the animals injected with the influenza strains, was 0.5 c c for each 100 gm. of body weight. The weight of the guinea-pigs ranged from 320 to 400 gm., the average being 330 gm. Intraperitoneal injections were made in 18 guinea-pigs with the 14 different strains of type pneumococci, 6 with 6 strains of type I; 5 with 3 strains of type II; 6 with 4 strains of type III, and 1 with 1 strain of group IV. Eleven of the animals died on the first day and 7 on the second day after injection, a mortality of 100% (table 2). The blood and peritoneal exudate showed large or countless numbers of pneumococcus colonies in all these animals. The peritoneal exudate in most instances and the blood in some instances yielded in addition a small and variable number of staphylococcus colonies. The volume of the lungs ranged from 4 to 9 c c, averaging 7 c c. Noteworthy lesions in the lungs were absent in all. A few showed small hemorrhages. A beginning pleuritis was noted in 5.

* For the strains of pneumococci used in these experiments I am indebted to Dr. Rufus J. Cole of the Hospital of the Rockefeller Institute for Medical Research and Dr. Augustus Wadsworth of the New York State Board of Health.

Successful intratracheal injections were as follows: 7 guinea-pigs with 5 strains of type I; 4 with 3 strains of type II; 5 with 4 strains of type III, and 4 with 2 strains of group IV pneumococcus, a total of 14 strains and 20 animals (table 2). Respiratory embarrassment immediately after injection and the following day was relatively slight as compared with animals injected with the influenza strains. In 7 of those that recovered no noticeable increase in respiratory rate or illness could be detected the day after injection. Five of the others showed increased respiration and rise in temperature for a day or two, and then recovered. Two showed marked increased respiration for 4 days and when improving, on the fifth and sixth day, respectively, were chloroformed. The findings in the former are illustrated in the experiment in guinea-pig 1,450. The other showed a resolving pneumonia with a few pneumococci in the blood and lung exudate. The 6 animals that died (one each injected with types I, II and III, and 3 of group IV pneumococci) showed with one exception progressive increase in respiration rate until death. The one that did not, died eight days later with a resolving pneumonia. The respirations in the 3 that died within five days after injection were extremely rapid, the nostrils remained free from exudate, there was no bleeding from the nose, the animals were quiet, and the breathing was generally free and easy. The picture was thus in sharp contrast to that noted in guinea-pigs that died following intratracheal injection of the highly virulent influenza strains. The tendency to the production of leukopenia following injection of type pneumococci even in fatal infection was far less than that following injection of the influenza strains, occurring respectively in 11 and 57% of the animals injected. The lungs were moderately distended, but smaller than those observed following the injection of influenza strains. The consolidation usually involved whole lobes or was sharply outlined (fig. 4). The pleura overlying the consolidated area was opaque and rough, the consolidated areas, even in the stage of red hepatization, were less edematous than in the influenzal lungs, and the areas showing gray hepatization were uniformly dry and granular. Extensive gray hepatization was noted as early as forty-eight hours after injection. Sections in the early stages showed many red cells and moderate leukocyte infiltration in the alveoli. Later diffuse alveolar infiltration with leukocytes and fibrin occurred with a few red blood corpuscles and little edema. The bronchial and alveolar epithelium showed comparatively little damage (figs. 18a and 19b). The pneumococci were found diffusely distributed in large

DERIVATION OF THREE FILTRATES AND THE RESULTS FOLLOWING THEIR INJECTION



numbers throughout the exudate and showed little tendency toward peripheral aggregation along the alveolar lining, and the perivascular and subpleural spaces (fig. 18b). There was no distinct difference in the exudate in animals injected with the different type strains. The findings in two experiments will suffice to illustrate:

Guinea-pig 1031, weighing 400 gm., was injected intratracheally, Jan. 22, 1919, with 1.5 c c of glucose-broth cultures of pneumococcus group IV after one (intraperitoneal) animal passage. The temperature before injection was 102.4 F., and the white blood count was 13,200. The following day the animal appeared quite well. The temperature was 99.6 and the white blood count was 18,600. January 24 the animal was found dead. The white blood count was 17,500. Most of the right lung was consolidated, uniformly grayish-red, firm in consistency, and quite dry on the cut surface. A number of smaller areas of consolidation were found in the left lung. The cut surface everywhere was fairly dry and nowhere could edematous fluid be made to drip from it. Emphysema was moderate. The total volume of the lungs was 10 c c. The cultures from the blood and lungs showed large numbers of green-producing pneumococci.

Guinea-pig 1448, weighing 380 gm., was injected intratracheally, Oct. 21, 1918, with 1.5 c c of glucose-blood-broth culture of pneumococcus type II (Strain 3625). October 22 the respirations were extremely rapid and the animal sat quietly. October 23 it was dead. A moderate amount of slightly turbid fluid was found in both pleural cavities. The lungs were moderately distended (14 c c) and weighed 10 gm. The right diaphragmatic lobe was quite uniformly gray and completely consolidated; it was dry and granular on the cut surface. A number of smaller areas of hemorrhage with decided consolidation were found chiefly around the bronchi in the left diaphragmatic and right cardiac lobes. There were marked myocardial degeneration and cloudy swelling of the kidneys, but the uterus and other organs were normal. Cultures from the blood yielded many pneumococcus colonies. Sections of the lung showed moderate distention of alveoli, absence of necrosis of alveolar epithelium and capillaries, and marked, highly cellular leukocytic infiltration of the alveoli in which large numbers of diplococci were distributed throughout the exudate with little tendency of the bacteria to be distributed along the alveolar epithelial lining (figs. 18 and 19 b).

PROTOCOLS OF CASES OF INFLUENZA AND INFLUENZAL PNEUMONIA AND ANIMAL EXPERIMENTS. SIMILARITY OF LOCALIZATION OF MICRO-ORGANISMS

Case 2607, a middle-aged woman developed pneumonia during an influenzal attack and died. The sputum obtained Nov. 21, 1918, was bloody; smears showed large numbers of gram-positive, lanceolate diplococci, gram-positive cocci, and small gram-negative bacilli of irregular size resembling influenza bacilli, and large numbers of gram-positive diplococci, at times in chains within epithelial cells. Blood-agar plates showed large numbers of colonies of green-producing streptococci and influenza bacilli. The sputum (0.3 c c) was injected intraperitoneally November 21, into Guinea-pig 737. November 22 at 8 a. m. the animal appeared to be ill, was irritable and short of breath. At noon it was worse. The respirations were greatly increased and it had repeated choking spells resembling anaphylactic shock. At 8 p. m. it was found dead, and was examined at once. A small amount of turbid fluid without fibrin was

found in the peritoneal and pleural cavities. The lungs were distended (13 c c), hyperemic and edematous, and showed numerous small hemorrhages and a number of large subpleural hemorrhages posteriorly in the left diaphragmatic lobe (fig. 5). In cultures from the blood were a few green-producing streptococci; the hemorrhagic area in the lung and peritoneal fluid contained large numbers of green-producing streptococci in pure culture. No influenza bacilli were found in smears from the peritoneal fluid. Sections of the lung showed marked congestion of interalveolar capillaries, marked hemorrhage in the alveoli, and desquamation and necrosis of the alveolar epithelial cells in varying degree. Many alveoli and terminal bronchi were greatly dilated; others appeared to be collapsed. The larger bronchi were constricted and their lumen contained numerous red blood corpuscles and desquamated alveolar epithelium; the mucous membrane lay in great folds. The hemorrhagic areas were usually situated around bronchi and beneath the pleura. In the latter position they were often triangular in shape with the base toward the pleura. At no place was there marked leukocytic infiltration (fig. 10). Prolonged study of sections stained by Gram-Weigert and by the combination of Goodpasture and Weigert stains recommended by MacCallum showed an interesting distribution of the bacteria. None were found within capillaries and larger blood vessels. A few were found in the areas of hemorrhage in the alveoli, but by far the largest number were found, as shown in figure 11, just outside the capillary in the interstitial tissue of the alveolar wall (a), along the alveolar lining beneath the desquamated epithelium (b), in the epithelial cells showing poorly stained nuclei, but still in place lining the alveoli showing hemorrhage (c), and in the degenerated, desquamating alveolar epithelial cells (d).

The streptococcus from the peritoneal fluid in this guinea-pig in the third culture generation was injected intraperitoneally into another guinea-pig. It died in twenty-four hours with turbid hemorrhagic fluid in the peritoneal cavity and numerous hemorrhages in a large part of the secum, especially surrounding the lymph follicles, and hemorrhages in Peyer's patches, but with no lesions of the lung. Intraperitoneal injections of the sputum in 3 other normal guinea-pigs was followed by the death of all in from three to ten days. The animal that died in 3 days was found to have emphysema, hemorrhages and edema of the lungs. The others showed no lesions of the lung. Two guinea-pigs injected two weeks previously with the sputum from other cases of influenza recovered.

The points of particular interest in these experiments are the marked affinity of the streptococcus in the sputum for the epithelium of the alveoli of the lung (fig. 11), the noninvasive power of the influenza bacilli found in the sputum, the hemorrhages in the intestine in the second animal passage, and the acquired immunity in the two guinea-pigs previously injected with sputum from other cases of influenza.

Case 2769, Miss M. J., aged 38, came for examination on account of chronic loseness of bowels and loss of weight and strength. Her condition was found to be due to pancreatic insufficiency. The patient contracted influenza Dec. 16, 1918; developed symptoms and signs of bronchopneumonia December 22, and died December 28. The looseness of bowels was worse throughout the influenzal attack. At necropsy were found "a resolving 'lobar' pneumonia, seropurulent pleuritis (1,500 c c), of the right side, chronic parenchymatous and interstitial pancreatitis, and fatty degeneration of kidneys."

Cultures from the lung after death and pus from the right pleura showed many hemolytic streptococci and a few staphylococci. The primary culture in glucose broth from the lung was injected intratracheally into one guinea-pig

and intraperitoneally into another. The guinea-pig (Guinea-pig 875) injected intratracheally died nineteen days after injection from hemorrhagic and purulent pleuritis, pericarditis and myocardial degeneration (fig. 7). The animal injected intraperitoneally died after twenty-four hours. It had diffuse peritonitis, extreme hyperemia of the large and small intestines, swollen lymph follicles throughout the intestinal tract, and numerous hemorrhages in the lower two thirds of the small intestine and in the cecum. The contents of the small intestine consisted chiefly of bloody mucus. The duodenum, stomach, and suprarenals were normal. The lungs were emphysematous (11 c c), and showed moderate edema and a number of small hemorrhages. The pleural cavity contained a small amount of hemorrhagic fluid. Cultures from the blood, peritoneal fluid, and intestinal contents showed hemolytic streptococci and staphylococci. The pleural exudate (1 c c) was injected directly into the trachea of a guinea-pig. It aborted four days later and died with marked leukopenia, increased respirations, voluminous lungs (17 c c), marked hemorrhagic pleuritis, hemorrhagic bronchopneumonia and lesions in the psoas muscles. Large numbers of green-producing streptococci were isolated from the lung, pleural fluid, and uterus but none from the spleen, liver, suprarenals and cervix. The culture from the peritoneal exudate was injected intravenously into a rabbit and one guinea-pig, and intratracheally into one guinea-pig. The rabbit died the day following injection with extreme distention of the abdomen due to a large amount of gas (rich in carbon dioxid) in the small intestines. The small and large intestines contained a large amount of mucus. The intestinal contents were liquid or semisolid throughout. The intestinal wall was opaque, but there were no hemorrhages. Six sharply circumscribed hemorrhages were found in the mucous membrane of the cardiac end of the stomach; in the medulla of the kidneys were a few embolic hemorrhagic areas. The myocardium was markedly degenerated. Cultures from the blood showed a large number of hemolytic streptococci and staphylococci. The guinea-pig injected intravenously died five days later. It showed two small areas of bronchopneumonia, swollen Peyer's patches and solitary lymph follicles, a large amount of mucus in the intestines, absence of food in the stomach, but a large amount of turbid mucus showing many gram-positive diplococci, edematous and hemorrhagic mesenteric lymph glands, marked hyperemia of the uterus, turbid mucus in both uterine horns, numerous small hemorrhages in the mucous membrane of the uterus and cecum, and focal lesions in the medulla of the kidney. Cultures from the blood showed one colony; cultures from the pneumonic areas, countless numbers, and from the mucus from the left horn of the uterus, a moderate number of staphylococcus colonies.

The guinea-pig injected intratracheally died six days later. It had voluminous lungs (15 c c), bronchopneumonia and edema of the right and left caudal lobes, hemorrhagic tracheobronchial lymph glands, purulent material in the nostrils, purulent bronchitis and tracheitis, a hemorrhagic fetus in the vagina and one still attached to the uterus; focal lesions in the medulla of the kidneys; and edematous mucous membranes of the pelvis of the kidneys. Cultures from the pneumonic lung and blood showed green-producing streptococci and staphylococci.

The striking features in the animal experiments in this case was the tendency to produce, in addition to the characteristic lung lesions and pleuritis in the first animal passage, lesions of the intestinal tract and medulla of the kidney in the second animal passage.

Case 2770, Mr. S. M., aged 33, was admitted to the isolation hospital Dec. 24, 1918, complaining of severe weakness, backache, aching all over, extreme nervousness and severe cough. These symptoms had begun two days previously. The leukocyte count the day of admission was 3,800. The temperature was 103 F., pulse 118, respirations 28. The patient grew progressively worse, the temperature ranging between 103 and 105. December 26 evidence of involvement of the lungs became apparent. Cyanosis and dyspnea increased as evidence of a rapid filling of the lungs appeared and the patient died December 28, forty-eight hours after the first signs of pneumonia had developed. Necropsy showed voluminous lungs, pseudolobar pneumonia associated with marked hemorrhagic edema involving all lobes, a large accumulation of bloody, turbid fluid in the left thorax (900 c c) and intense hemorrhagic bronchitis. In cultures made from the sputum December 26 were enormous numbers of green-producing streptococci and a few staphylococci; in the lung exudate and blood after death were many green-producing streptococci and staphylococci.

The primary culture in glucose broth from the blood of this patient was injected into the trachea of 3 guinea-pigs in doses of 0.1 c c, 1 c c and 2.5 c c, respectively. The one receiving only 0.1 c c had moderately increased respirations for several hours, then appeared quite well for four days, when the respirations again became rapid and the animal died six days after injection with a moderate amount of hemorrhagic turbid fluid in the pleural cavity, moderate distention of the lung (12 c c) with almost complete consolidation of the left anterior and cardiac lobes. The cut surface of the consolidated areas was mottled grayish-red and edematous between the areas of denser consolidations and necrosis. Cultures from the blood showed green-producing streptococci in pure culture; those from the lung and pleural fluid, green-producing streptococci and staphylococci. The leukocyte count was 17,000 before injection, 8,960 four hours after injection, and 5,200 the following day. The animal injected with 1 c c died in five days after having aborted. It showed acute diffuse peritonitis clearly secondary to infection in the uterus which passed through the left tube, voluminous lung (15 c c), hemorrhagic bronchopneumonia, pleuritis, and marked maxillary sinusitis, tracheitis, and bronchitis. The animal injected with 2.5 c c died in four and one-half hours. It had extreme difficulty in breathing and frequent paroxysms resembling anaphylactic shock; it was found with its head in a pool of hemorrhagic edema fluid. The lungs were voluminous (20 c c), hemorrhagic, and edematous throughout. The trachea, bronchi, and nostrils were filled with hemorrhagic frothy fluid.

The glucose-brain-broth culture of green-producing streptococcus derived from a single colony on the blood-agar plate from the blood in this case was injected into 3 guinea-pigs; all received 1 c c intratracheally. One of these had had an intraperitoneal injection of a primary culture from the sputum in another case of influenza 10 days previously. It showed no symptoms the day after injection and remained well subsequently. One of the others died the day after injection with leukopenia, voluminous lung (12 c c), marked hemorrhagic edema and bronchopneumonia, edematous peribronchial lymph glands, and a moderate amount of fluid in the pleural cavity. The third died in 3 days of hemorrhagic bronchopneumonia, tracheitis, and sinusitis. Both of these showed green-producing streptococci in the blood and green-producing streptococci and a few staphylococci in the lung and pleural fluid. The glucose-brain-broth culture injected into these 3 guinea-pigs was subcultured rapidly in duplicate from tube to tube of glucose-brain broth, and cultures made from one to three times a day. In the eleventh subcultures 2 guinea-pigs were injected intratracheally with 1 c c and 1.5 c c, respectively, of the two cultures.

Both were found dead the following day. The blood-agar plates of the culture injected showed a pure culture of staphylococci and smears showed the absence of streptococci. The lungs in both were hemorrhagic and edematous but were compressed (5 c c) by a huge accumulation of chocolate-colored fluid in the pleural cavities (20 c c in each). Both had hemorrhagic placental masses in the uterus; several were detached and being expelled (fig. 9). Sections of both showed large numbers of staphylococci throughout the lungs, especially beneath the pleura, and no streptococci. Cultures from the blood of both showed staphylococci; from the lung, pleural fluid, and hemorrhagic placental masses, large numbers of staphylococci and a few colonies of green-producing streptococci; and from the liver, kidney, and ovaries a small number of staphylococci. The symptoms in the guinea-pig (Guinea-pig 940) injected with 1.5 c c were noted for nine hours prior to death. Respiratory embarrassment at first consisted chiefly of difficulty in expiration; later breathing became easier but exceedingly rapid as from a filling thorax. The animal was examined immediately after death in order to note the condition of the uterus. Violent waves of uterine contraction continued for some minutes. One of the placental masses was partially detached; all were hemorrhagic. The hemorrhagic pleural fluid was immediately injected into the trachea of another guinea-pig, which showed moderately increased respirations immediately after injection, seemed well the following day and remained so for twenty-six days, when it was chloroformed. The pericardial sac was thickened and distended with bloody fluid. The peribronchial and mediastinal lymph glands were edematous and much enlarged. The pleura and lungs were normal. Cultures from the pericardial fluid and lymph glands showed staphylococci.

The points of special interest in the experiments in this case are the high virulency of the strain isolated from the blood, the tendency to produce the same type of lesions over a wide range of dosage, the immunity induced by a previous injection of a culture from influenzal sputum, the extreme contractions of the uterus, and the marked infectiousness of the culture, showing what seems must be considered as a mutation of green-producing streptococcus into staphylococcus.

Case 2787, a man, aged 59, had influenzal pneumonia and pleuritis from which he made a slow recovery. There was little expectoration. A diagnostic puncture of the chest was made Jan. 8, 1919. A small amount of turbid, bloody fluid was aspirated which showed countless numbers of colonies of hemolytic streptococci in pure culture. A suspension in salt solution of one-thirtieth and one-third of the primary growth on a blood-agar plate was injected into the trachea of 2 guinea-pigs, respectively. The former had increased respirations for several days and then recovered; the latter had increased respirations for several days and died two weeks later of abscess and gangrene of the right diaphragmatic lobe, pericarditis, and pleuritis. The pericardium was markedly thickened and distended with gelatinous organizing, fibrinous exudate. The pleural cavity contained a large amount of foul smelling pus communicating with the abscess (fig. 8). Cultures from the blood showed no growth; the pericardial fluid showed staphylococci, the pleural fluid, staphylococci and gram-negative bacilli.

Case 2798, Mr. E. C. B., aged 26, was admitted to the isolation hospital Jan. 7, 1919. He had been taken ill seven days before with cough, general malaise, sore throat, and chills, but he did not ache severely. The temperature on admission was 103 F., but it dropped to normal the following day. The leukocyte count January 8 was 11,400; January 10, 12,500, and January 11, 14,300. The patient had a moderately severe cough in which he raised muco-

purulent sputum. No definite chest signs could be detected on physical examination, but the roentgen-ray examination on the day of admission showed slight bronchial infiltration in the left lung, and January 14 a small area of infiltration in the right middle lobe. The patient was discharged from the hospital January 14, after the temperature had been normal for 5 days, although the cough persisted. January 16, he was again admitted to the hospital complaining of a sharp, severe pain in the right lower chest aggravated by breathing, of malaise, and of feeling weak generally. At this time he had fever for five days; he developed outspoken signs of pleuritis over the right side of the chest, and pleural thickening over this area was manifested by roentgen examination January 26. The sputum obtained January 11 showed countless numbers of colonies of hemolytic streptococci, a few green colonies of streptococci, small indifferent colonies of influenza bacilli, and a number of staphylococci.

The culture in glucose brain broth from a single colony of hemolytic streptococcus (which yielded a pure culture of green-producing streptococci on blood-agar plates) was injected into the trachea of Guinea-pig 957, January 13. The leukocyte count was 16,000 and the temperature 102.4 F. The following day the animal seemed ill, respirations were rapid, the temperature was 97 and the leukocyte count was 19,000. On the second day the symptoms were about the same, the temperature was 103.6, and the leukocyte count was 16,500. January 16 the temperature was 102.8, the leukocyte count was 16,500. January 16 the temperature was 102.8, the leukocyte count was 17,000, the respirations were definitely increased, and the animal appeared sick. January 19 it was found dead. The pleural cavity was distended with a large amount of bloody, chocolate-colored fluid, partially walled off in pockets with fibrinous adhesions, and partially obliterated by fibrinous adhesions, and the visceral and parietal pleura and the pericardium were covered with a thick layer of fibrinous material (fig. 7). The lungs were moderately distended (14 c c), moist and edematous on the cut surface, but consolidation was limited to several small areas. Cultures from the blood, pleural fluid, and lung showed a large number of green-producing streptococci and some staphylococci; from the spleen and kidney, a number of green-producing streptococci, and from the liver and suprarenal, no growth.

The attack in this case of influenza was atypical; there was no reduction in leukocytes, and the attack occurred during a quiescent interval between two epidemic waves. The point of special interest is the fact that the findings in the animal injected with a culture from the sputum paralleled the findings in the patient quite accurately in that leukopenia did not occur; the lung lesions were slight, and the involvement of the pleura was the marked lesion.

Case 2809, M. D., a little girl, aged 3, was admitted to the isolation hospital Jan. 8, 1919, in a weak condition with a temperature of 103.4 F., pulse 152, respirations 32, moderate cyanosis, and a severe cough. She had been taken sick that day, and was running a typical course of influenza without apparent lung involvement. The temperature ranged between 101 and 102 degrees for four days, becoming normal on the fifth day. The throat was moderately red; the tonsils were normal, the tongue coated. The day after admission it was noted that the vulva was inflamed and that pus was discharging from the vagina. The condition yielded promptly to irrigations and douching with a weak solution of potassium permanganate. January 12 smears of the vaginal discharge showed a moderate number of leukocytes, many gram-positive, lanceolate diplococci, often in short chains, gram-negative bacilli, some resembling Bacillus coli and many smaller gram-negative bacilli resem-

bling Bacillus influenzae. Blood-agar plates showed a large number of green-producing streptococci, a moderate number of colonies of colon bacilli, and many small colonies resembling Bacillus influenzae. The colonies of the latter were most numerous and the growth more luxuriant immediately surrounding the colonies of the streptococci. Smears of these small indifferent colonies showed gram-positive and gram-negative small bacilli or short-chained diplococci. A subculture on a blood-agar plate of a single colony of the green-producing streptococcus, including some of the small indifferent colonies, yielded pure growth of green colonies of streptococci; of four single colonies resembling Bacillus influenzae no growth was obtained, whereas subcultures from a group of these colonies yielded countless numbers of influenza bacillus-like colonies and a moderate number of green streptococcus colonies. Subcultures in two bottles of glucose-blood broth from the groups of isolated Bacillus influenzae colonies, well separated from green colonies of streptococci, yielded countless numbers of streptococci, blood-agar plates from these showing countless colonies of green-producing streptococci.

The primary culture in glucose brain broth from the vaginal swab was injected into the trachea of 2 guinea-pigs. Both pigs died within twenty-four hours of markedly dilated lungs filled with acute hemorrhagic edema fluid; both developed marked leukopenia, abortion with hemorrhage in the uterus, and both showed gram-positive diplococci in sections of the hemorrhagic edematous areas in the lung (fig. 17). A pure culture of the green-producing streptococcus in glucose brain broth in the second generation was injected into the trachea of 2 guinea-pigs. The female died within forty-eight hours with marked reduction in leukocytes, massive hemorrhagic bronchopneumonia, edematous mucous membrane of the uterus, cervix and vagina, hemorrhages in the cervix and upper portion of the vagina, and a large amount of turbid mucus in the vagina. Smears from mucus in the vault of the vagina showed a moderate number of gram-positive diplococci and a few large gram-negative bacilli. The male recovered and showed moderate reduction in leukocytes; he had fever for several days, but no other noteworthy symptoms.

The streptococcus isolated in this case, in addition to causing characteristic lesions of the lungs, showed marked affinity for the uterus and vagina; it was agglutinated specifically by the monovalent antistreptococcus serum.

Case 3171, Mr. P. H. L., aged 30, was taken with headache, severe aching all over and chilliness Feb. 26, 1919. He felt so sick that he was obliged to go to bed. After resting for a number of days he felt better, but March 2, after a hearty meal, he developed high fever, cough, and a headache with sweating. The following day cyanosis, rapid respirations, crepitant râles and bronchial breathing over the left lower lobe were noted. The next day numerous moist râles were heard over the right lower lobe, and the respirations were labored. The sputum became serous, bloody, and frothy March 5, and the patient died March 6. At necropsy marked bilateral hemorrhagic pneumonia of the greater portion of both lungs and marked hemorrhagic tracheobroncho-bronchiolitis were found. Histologic examination of the lungs showed marked congestion of the alveoli which were filled with edematous exudate containing few cells.

Cultures from the mucopurulent material in the larynx, trachea, and bronchi showed numerous spreading, slightly hemolyzing streptococci, a moderate number of staphylococci, and hemolytic streptococci. Cultures from the pleural fluid showed a large number of green-producing streptococci and from the glucose broth inoculated with the blood, green-producing streptococci. The primary culture in glucose broth was injected into the trachea of male G. pig

1249. The respirations the following day were extremely rapid and labored, the animal appeared ill, and was found dead the next day. The lungs were huge, 26 c c, and extremely heavy, 21 gm. A large amount of bloody, edematous fluid ran from the cut surface. The whole left lung appeared uniformly consolidated, and most of the right lung showed irregular areas of consolidation, emphysema, and hemorrhagic edema (fig. 3). There were no other lesions. Cultures from the blood, lung, and spleen showed many large, moist, spreading, green colonies of streptococci.

The primary culture in glucose broth from bronchial exudate was injected intraperitoneally into a mouse and into the trachea of a female guinea-pig. The mouse died of peritonitis within twenty-four hours. The guinea-pig died of hemorrhagic bronchopneumonia, hemorrhagic pleuritis, and a hemorrhagic infection of three fetuses with abortion, in forty-eight hours. The cultures showed large numbers of colonies of typical hemolytic streptococci and a moderate number of staphylococci. The primary culture of the pleural fluid was injected into a male guinea-pig. It appeared ill the following day and sat humped up; its hair was rough, and the voice was hoarse. It gradually improved during the following four days and remained well.

The results in this case, in addition to the production of the characteristic lung and uterine lesions, are of interest because of the bacteriologic findings in the blood, pleural fluid and the lung exudate, and in showing changes in the character of these organisms as they were passed through animals, the green-producing streptococcus from the blood acquiring the property of producing large, spreading, moist, green colonies, and the spreading, slightly hemolyzing streptococcus from the bronchial exudate becoming a typical hemolytic streptococcus.

Case 3175, Mrs. C. S., aged 24, was operated on March 1, 1919, on account of recurring attacks of appendicitis which were considered sufficiently serious to warrant operation for the removal of the appendix even though she was four months pregnant. The patient did well for five days and then developed fever, cough, dyspnea, mucopurulent and bloody sputum, rapidly progressing pneumonia, and pleuritis of the right side. She aborted on the seventh day after the operation and died from acute pulmonary edema on the eighth. Necropsy was refused. The sputum obtained March 7 was purulent and chocolate colored. The blood-agar plate showed countless numbers of staphylococci.

The human fetus (male) was brought to the laboratory while fresh; the membranes had not ruptured. The trunk measured 10 cm. in length. There was marked edema in the anterior cervical region surrounding the trachea and in the left abdominal rectus muscle. The left pleural cavity was free from fluid; the right contained a large amount of turbid hemorrhagic fluid fully 5 c c. The pericardial sac contained a small amount of turbid fluid free from blood. Blood-agar plate cultures from the brain, intestinal contents, pericardial fluid, and edema fluid from the subcutaneous tissue remained sterile. Glucose brain-broth cultures of hemorrhagic fluid from the right pleural cavity, pericardial fluid, and subcutaneous edema fluid showed short-chained streptococci and staphylococci. Sections of brain, kidneys, suprarenals, liver, and spleen showed no noteworthy lesions and no bacteria. Sections of the right lung showed a moderate number of diplococci in the pleura and subpleura of the right side (fig. 22).

The sputum containing streptococci in addition to staphylococci was injected into the trachea of 3 female guinea-pigs. All died within twenty-four hours from hemorrhagic edema of the lung, and hemorrhagic pleuritis, and all aborted.

Two showed interstitial pulmonary emphysema, one in the form of pleural blebs only, the other in frothy fluid in the pleural cavity due to rupture of one of these blebs. Cultures from all yielded staphylococci and streptococci. In order to determine the infecting power of the staphylococcus, the culture in glucose blood broth from a single colony was injected into the trachea of another female guinea-pig. It died within twenty-four hours from hemorrhagic edema, hemorrhagic pleuritis, and abortion, and showed staphylococci in the blood and pleural fluid in pure culture. Sections of the lung of one of these pigs showed the characteristic hemorrhagic edema with slight cellular infiltration and large numbers of streptococci and staphylococci in the lung (fig. 21).

A large white and gray cat (cat 24), with advanced pregnancy, a leukocyte count of 11,600 and a temperature of 101.4 F., was injected in the trachea March 17, 1919, with 4 c c of glucose-blood-broth culture of streptococcus in the second culture from one of the guinea-pigs. March 18 the temperature was 101.4, the white count 6,600, and the animal appeared well. March 19 and 20 it appeared quite well but refused food, and the temperature was 102.2. March 21, 12 m., it was found in labor; one kitten was born. By 6 p. m. 4 kittens had been born; all were alive and appeared nearly full-time size. March 22 the cat and kittens appeared well, but the cat refused food; the kittens nursed. March 23, one kitten was found dead; the cat refused food and appeared sick. March 24, another kitten was found dead, and a third was sick. The cat was worse, refused food, the respirations had increased, and a vaginal discharge of bloody pus had developed. March 25 the third kitten was dead and the fourth was in a dying condition. The cat, which was so weak that she was just able to stand, and had a marked diarrhea and a temperature of 104, was chloroformed. The uterus showed large numbers of small submucous hemorrhages; the mucous membrane was swollen in places, necrotic, and covered with a thick layer of chocolate-colored pus. The liver showed marked fatty degeneration, the kidneys, acute nephritis. Smears from the uterine exudate contained many staphylococci and streptococci. In cultures from the uterus were countless numbers of colonies of staphylococci and green-producing streptococci and number of spreading gram-negative bacilli; from the blood, liver and spleen, a few colonies of staphylococci and streptococci; from the suprarenal and kidney, no growth. Sections of the uterus showed a thick layer of leukocytes covering the endometrium, containing enormous numbers of staphylococci and streptococci. Fetus 1 showed no lesions. Cultures from the blood showed large numbers of stphylococci and a few green streptococci. Fetus 2 was accidentally discarded by the animal keeper. Fetus 3 showed 5,800 leukocytes in the blood and a small amount of blood-tinged pleural fluid, staphylococci, and a few green streptococci in the blood and pleural fluid, together with a spreading gram-negative bacillus. Sections of the lung showed gram-positive diplococci in the subpleural space duplicating the findings in the human fetus (fig. 22). Fetus 4 showed no gross lesions, but the same organisms in blood and urine as in fetus 3.

The points of particular interest in this case are the invasion of the fetus with lesions of the pleura caused by the organisms found in the sputum, the production of marked lesions in the lung and pleura, including interstitial emphysema of the lung in guinea-pigs, the marked affinity for the placenta, the invasion with the production of pleural lesions of the fetuses in the cat, and the sensitiveness of the streptococcus to oxygen.

The findings in the patients and the results obtained in each case reported herewith in detail are quite accurately representative of the findings of the whole series studied. I have purposely included one case (case 2787) in which leukopenia was absent and in which the diagnosis of influenza was doubtful because the results in the animal injected paralleled so accurately the findings in the patient. The points noted which deserve special emphasis are the marked affinity of the streptococci for the epithelium of the lung, even after intraperitoneal injection (fig. 11), the occurrence of characteristic lesions of the lung and pleura, the frequent involvement of the uterus resulting in abortion, and the very great similarity of the results obtained in the animals and the findings in cases 2769, 2770, 2787, 2798, 2809 and 3175. The findings in the little girl who developed vaginitis during her influenzal attack leave little doubt that the vaginal discharge was the result of localization and infection by the streptococcus in the uterus and vagina. The localization of the streptococcus in the pleura of the fetus (fig. 22) of the pregnant patient (case 3175), and in the pleura of the fetuses in the pregnant cat injected into the trachea with the sputum of this case, when absent in other tissues, may be regarded as elective localization of bacteria of high order. On the basis of these findings a study of the tissues of fetuses and of the new-born said to have contracted influenza in utero should be undertaken, since the micro-organism found in the affected tissues under these conditions may be considered responsible for the production of the lesions, and thus add materially to our knowledge of the etiology of influenza.

EXPERIMENTS WITH FILTRATES OF LUNG EMULSIONS AND CULTURES

After it was noted that intratracheal application of the bacteria from patients with influenza had marked effect it was thought that this method of injection might prove valuable in studying the effects of filtrates of material from influenza. Exceedingly small forms of diplococci were frequently seen in smears of sputum, in throat swabs, and in cultures from influenza, especially in deep tubes of glucose brain broth. It was thought possible that the preformed toxic products in filtrates of cultures and lung emulsions might injure the respiratory epithelium and thus facilitate growth of the few organisms which might pass through the filter, and, since the lung appeared to be the point of predilection of these organisms, growth might occur when cultures were introduced in this manner even though control cultures

on artificial mediums were negative. Moreover, valuable light might be thrown on the question of the presence or absence of a filtrable virus in this disease. I wish here to summarize the experiments done along this line. The filtrates studied were from sputum obtained, early in the course of the disease, from the lungs of dead animals, with the characteristic picture following injection of sputum or cultures from sputum, and from cultures from the sputum during life and from the blood and lung exudate after death of patients who had died from influenza.

Berkefeld N filters, Mandler filters, and dense unglazed porcelain filters were used. The filtrates from the lungs were obtained by making an approximately 10% emulsion of the pneumonic or hemorrhagic lung tissue in salt solution, centrifuging it fractionally, and filtering the opalescent fluid by the aid of a partial vacuum obtained with a water suction pump. The cultures of influenzal material in the tall tubes of broth were incubated twenty-four to seventy-two hours and filtered without centrifugation. The efficiency of the filters used was controlled with Bacillus prodigiosus, and they were found to remove these small organisms in every instance. Cultures from the filtrates were made on blood agar, and in deep tubes of glucose brain broth and in tissue broth. The brain, which weighed approximately 1.5 gm., was added to each tall tube of glucose broth before autoclaving; the tissue (rabbit kidney) was added in a sterile manner to meat infusion broth sterilized by the fractional method. The inoculations in these mediums were made with at least 0.5 c c of the filtrate. The tubes were incubated at 33 to 35 C. for a week before they were discarded. Altogether, 15 filtrates have been made and studied. The cultures on blood agar were negative in all. Those in broth remained free from growth in 13, while 2 (filtrates 6 and 7) yielded definite growths. In the former, filtration was slow; in the latter, rapid. In 4 of the broth cultures clouding was distinct, but smears and subcultures were negative.

The animal experiments consisted of intratracheal injection of the usual dose of the filtrate itself, of the "negative" cultures in the broth, and in some instances of the respective culture and lung emulsion as controls. Altogether, 42 guinea-pigs were injected intratracheally, 30 with the filtrate directly, and 12 with the "negative" cultures from filtrates. Of these, 13 died; 10 were anesthetized for examination, and 19 recovered. The immediate symptoms following injection of the filtrates were indistinguishable from those following injection of the cultures. Death from fatal infections occurred somewhat later fol-

lowing injection of the filtrates than following injection of the corresponding cultures or emulsions. The lesions were similar. Emphysema, hemorrhage and edema of the lung, with coalescing areas of lobular pneumonia and hemorrhagic pleuritis, were striking features. Microscopically dilated alveoli filled with blood and edema fluid with marked desquamation and destruction of the epithelium of the alveoli and bronchi with relatively slight leukocytic infiltration formed the dominant picture. Of the cultures from the 13 guinea-pigs that died, 8 showed green-producing streptococci as the predominating organism, 2 showed Bacillus bronchisepticus in addition, 1 Bacillus coli, and in 1 the cultures remained negative; cultures were not made from 1 guinea-pig. In the 2 that showed Bacillus bronchisepticus an old pneumonia, easily distinguished from the hemorrhagic lesions due to the injection, was present. Sections of the lungs showed gram-positive diplococci which were most numerous along the alveolar wall. The 10 anesthetized animals had the usual immediate symptoms, increased respirations for from two to three days. Two were anesthetized while the symptoms were severe and progressing. The findings in these were similar to those in the animals that died (guinea-pig 882, guinea-pig 885). The lungs of the rest were only slightly emphysematous; hemorrhagic edema with little infiltration in relatively small areas in the lung was noted in most of the animals. The cultures in these showed green-producing streptococci in 3, no growth in 6, and in 1 the cultures were contaminated accidentally. Symptoms in those that recovered were either absent the day after injection or consisted of increased respirations, lessened activity, and ruffled fur for several days.

Six animals were injected with heated filtrates; all showed the usual immediate symptoms of anaphylactic shock, somewhat less severe but otherwise comparable with the symptoms in animals injected with the corresponding unheated filtrate. They had no symptoms subsequently and all remained well.

Leukocyte counts were made in 26 animals injected with filtrates. In 17 a marked or decided drop in leukocytes occurred, in 2 a slight drop, while in 7 no noteworthy change occurred. None showed leukocytosis. The leukopenia was usually present twenty-four and forty-eight hours after injection, after which return to normal occurred in the animals that recovered; in those that died it usually persisted, and

sometimes the count became progressively lower until death (guinea-pig 888). The drop was not usually as marked as that following injection of cultures.

Leukopenia occurred following injection of filtrates from sputum, from lung emulsions, and from broth cultures of freshly isolated strains. Heating to 60 C. for thirty minutes and to the boiling point for ten minutes was found not to destroy the substance causing a diminution in leukocytes, nor did heating destroy the property causing immediate symptoms of anaphylactic shock.

Two filtrates that produced marked effects in animals on intratracheal injection were injected subcutaneously into 3 persons in doses of 4 c c for one, and 5 c c for each of the other two. All developed only slight local reaction and none fever or constitutional reaction. One person's throat was swabbed with a mixture of 2 filtrates, one prepared from the lung, and the other from a culture from the blood of case 2800. No symptoms followed. These filtrates (5 c c) were also injected subcutaneously in each of two persons. No symptoms occurred other than a negligible local reaction.

The results following injection of 3 filtrates (filtrates 2, 3 and 4) in a series of animals, and the derivation of the strain from which they were prepared, are summarized in the tabulation. Two guinea-pigs (guinea-pigs 908 and 909) injected with the fresh filtrate and the 2 (guinea-pigs 918 and 921) injected with the "negative" culture in glucose brain broth developed the characteristic symptoms and findings; 1 (guinea-pig 926) injected with the fresh filtrate remained well. The filtrate from the pneumonic lung of one of these (guinea-pig 908) was injected intratracheally while fresh into 4 guinea-pigs (guinea-pigs 930, 931, 933 and 934), and after being heated to 60 C. for thirty minutes was injected in 2 guinea-pigs (guinea-pigs 929 and 932). All showed decided immediate symptoms and 1 died in ten minutes of anaphylactic shock. Three of the rest had increased respirations for several days and then recovered, while the other 2 showed no symptoms. The filtrate from the pneumonic lung of guinea-pig 874 was injected into the trachea of 4 guinea-pigs (guinea-pigs 882, 888, 919 and 923). Three developed symptoms and died. The usual findings were noted.

Green-producing streptococci in pure culture, or together with staphylococci, were isolated from the characteristic lesions in all that died. Injection of the corresponding cultures into other guinea-pigs

was followed by very similar lesions (to be reported elsewhere). The parallelism was so striking that the mortality in the third animal passage (filtrate 3) was higher than in the fourth animal passage (filtrates 2 and 4). Sections of the lungs showed all the characteristic features peculiar to the influenza strains, including the localization of the streptococci. From the filtrate experiments it may be concluded that the green-producing streptococci from influenza in cultures and lung exudate may pass through filters through which Bacillus prodigiosus will not pass, and that they can multiply and grow when injected into the trachea of guinea-pigs, even though cultures remain negative.

Four protocols are given as illustrations:

Guinea-pig 921, weighing 260 gm., was injected intratracheally Jan. 6, 1919, with 1.5 c c of negative culture of the Berkefeld filtrate of the lung emulsion of Guinea-pig 851 (filtrate 3, see tabulation). January 7 the blood-agar plate from the nose made before injection showed a moderate number of colonies of Bacillus coli and staphylococci. January 8 the animal seemed ill and respirations were increased. January 9 and 10 there was marked increase in respirations and the animal seemed sick. January 11 it was found dead. The lungs were found moderately distended; their total volume was 9 c c. The diaphragmatic lobes were firm and heavy. The left lobe contained a whitish area of consolidation 1 by 0.5 cm.) along the margin surrounded by large areas of hemorrhage and edema. The cut surface of the lung was moist and a large amount of bloody fluid escaped. The peribronchial lymph glands were edematous. The uterus was small but showed a circumscribed area containing numerous punctate hemorrhages. On the flat muscles on the inner aspect of both thighs were numerous small whitish necrotic areas. The kidneys showed marked cloudy swelling. The left nostril and the corresponding sinus were filled with mucopus. The mucous membrane of the nose, trachea, and bronchi was edematous and congested. January 13 blood-agar plate cultures from the blood, the areas of consolidation, and edema fluid of the lung, the kidney, and mucus from the right and left horns of the uterus, contained large numbers of streptococci; emulsons of the muscles with lesions, contained 70 green colonies of streptococci; and the emulsions of the normal muscle, contained no streptococci.

Guinea-pig 908, weighing 350 gm., was injected intratracheally Jan. 4, 1919, with 1 c c of Berkefeld filtrate from Guinea-pig 851 (filtrate 3). The nostrils were dry at the time the cultures from the nose were made. January 5, at 11 a. m., the animal appeared to be quite well with slight increase in respiration. At 1 p. m. it suffered from a brief attack of shortness of breath and paroxysms of coughing; the voice was clear. At 5:30 p. m. the animal was hoarse and respirations were definitely increased. A violent attack of shortness of breath followed the making of a second culture from the nose. The blood-agar plate of secretion from the nose made before injection showed a large number of staphylococci and a few green-producing streptococci. January 6 a large number of staphylococci and green-producing streptococci were found in the cultures from the nose made the day after injection. At 9:45 a. m. the animal was short of breath. It had an attack resembling anaphylactic shock; the eyes were watery, the nostrils dry. At 3 p. m. the respirations were rapid and

an expiratory grunt had developed. At 8:30 p. m. the animal was very weak; respirations were extremely rapid and difficult, and it often made violent efforts to get its breath. It died in one of these paroxysms. The lungs were found to be extremely distended; their volume was 17 c c and the weight was 14 gm. A large part of both lungs was consolidated. The cut surfaces everywhere were extremely moist; a large amount of bloody, frothy fluid escaped and the trachea and bronchi were filled with a similar fluid. An uninvolved portion of the lung was extremely emphysematous and the alveoli appeared to be at the rupturing point. There were no areas of old lung lesions. The mucous membrane of the nose, the trachea, and the bronchi was hyperemic. The peribronchial lymph glands were edematous. The mucous membrane of the uterus was hyperemic. January 7, blood-agar plate cultures from the blood, kidney, liver, brain, and mucous membrane from the right and left horns of the uterus were sterile; in cultures from the pneumonic lung countless numbers of green colonies of streptococci were found; and from the nose and the mucous membrane of the turbinate bones, countless green colonies of streptococci and a large numbers of staphylococci. Smears from the lung showed large numbers of gram-positive diplococci and those from the nose, gram positive diplococci and staphylococci. No organisms resembling the influenza bacillus were found.

Guinea-pig 888, weighing 490 gm., was injected intratracheally Jan. 3, 1919, with 1.5 c c of the porcelain filtrate of the lung of Guinea-pig 874 (filtrate 2, see tabulation). Before injection the leukocyte count was 10,500. The nostrils were dry when cultures were made. The animal coughed violently several times while being injected and had shortness of breath for 15 minutes following the injection. The temperature rose to 103.4 degrees. January 6, the condition of the animal was about the same although the shortness of breath had increased somewhat. The cultures from the nose showed large numbers of green-producing streptococci and moderate numbers of staphylococci. January 9 the animal was found very weak and short of breath. It acted strangely, constantly pushing its head into the side of the basket or under its mate until it was completely exhausted. At 9 a. m. its temperature was 94; the white count 4,300. At 11 a. m. it died during a violent effort at respiration. The lungs were found distended (14 c c); there was an extensive bronchopneumonia, a mild pleuritis, four hemorrhagic areas, markedly recent placental attachments, one in the right and three in the left horn of the uterus. There was a large amount of turbid mucus in the uterus. January 11 blood-agar plate cultures of the blood were negative; those of the lung showed countless numbers of colonies of streptococci and a few staphylococci.

Guinea-pig 882, weighing 300 gm., was injected intratracheally Jan. 2, 1919, at 10 p. m. with 2 c c of porcelain filtrate of the lung emulsion from Guinea-pig 874 (filtrate 2). Cultures from the nose contained large numbers of staphylococci and diphtheroid bacilli. January 3 at 7:30 a. m. the animal appeared quite well, and without definite shortness of breath. At 11 a. m. there was definite shortness of breath. Much mucus was noted in the left nostril. This was cultivated and was found to contain a large number of green colonies of streptococci and a moderate number of staphylococci. January 4 respiration was increased and the animal had grown thin, weighing 260 gm. January 5 it seemed ill; the nostrils were moist; the temperature was 103.6. It was etherized; the lung was found moderately distended and the left anterior lobe completely consolidated, uniformly grayish-red, and mottled on the cut surface. The consolidated areas were moist and surrounded by hemorrhagic edema. The uterus contained a hemorrhagic area, one in each horn, and bloody mucus in

the fundus. In the right horn was found a chocolate-colored, hemorrhagic mass. January 7 blood-agar plate cultures of the blood showed a few green-producing streptococci; of the lung and nose, large numbers of green colonies of streptococci and a moderate number of Staphylococcus aureus; from edematous fluid of the lung, a moderate number of green colonies of streptococci; from the right horn of the uterus moderate numbers of green colonies of streptococci and one of staphylococci. Cultures from the liver and kidney were negative.

Guinea-pig 885, weighing 350 gm., was injected intratracheally Jan. 2, 1919, with 2 c c of Berkefeld filtrate with emulsion of the pneumonic lung of Guinea-pig 869. Cultures taken from the nose before injection showed staphylococci only. January 3 at 7:30 a. m. the animal appeared quite well; there was no apparent shortness of breath; at 11 a. m. the respirations were increased. Smears from the nose showed large numbers of diplococci, often in short chains. January 4 cultures from the nose made twelve hours after injection showed many staphylococci and green colonies of streptococci. The animal appeared sick, coughed and sneezed at intervals; respirations were rapid. January 5 shortness of breath had diminished. The animal was etherized. The lungs were moderately emphysematous (11 c c); one area of consolidation 1 by 0.7 cm. was found in the right diaphragmatic lobe. The pleura was dull over this area. The cut surface was markedly edematous and a large amount of bloody, frothy fluid escaped. A number of smaller areas of consolidation were found in the left diaphragmatic lobe. The peribronchial lymph glands were enlarged and edematous. There was mucopurulent material in the nostril. January 7, blood-agar plate cultures from the blood, liver, kidney and testicle were negative; those from the pneumonic lung showed large numbers of green colonies of streptococci, and those from the nose showed many staphylococci and green colonies of streptococci.

EXPERIMENTS INDICATING THE TRANSMISSION OF INFLUENZAL INFECTION BY CONTACT

The question of the possible transmission of infection by contact in the animals was also studied. Uninjected guinea-pigs and guinea-pigs injected with broth or salt solution were caged with animals inoculated intratracheally with cultures. All of the 8 uninjected pigs, and the 5 injected with salt solution remained well. In 5 of the former and 3 of the latter the nasal mucous membrane was injured with a sterile flexible wire coil when making cultures from the nose. Two of 10 guinea-pigs injected intratracheally as controls with glucose broth became ill with symptoms suggesting respiratory involvement. Both of these animals were caged with guinea-pigs injected with highly virulent cultures. One died in four days from hemorrhagic bronchopneumonia. Cultures from the blood and lung showed countless numbers of green-producing colonies of streptococci. The other died ten days after injection, with a large amount of a bloody fluid in the chest and marked bronchopneumonia. The blood contained green-producing streptococci and

the pleural fluid, staphylococci. The green-producing streptococcus from both guinea-pigs was agglutinated specifically by the monovalent serum. It corresponded morphologically and culturally with the streptococci from influenza and neither strain fermented inulin. During the course of these experiments the supply of normal guinea-pigs was large and no epidemic of pneumonia occurred. Examination of those that died spontaneously was made as a further check on the experiments. Five were found with lesions in lungs. These lungs were different in appearance from those that followed injection of the strains from influenza. They were small, the pneumonia process, usually old, was most marked in the anterior lobes instead of the posterior lobes, and the more recent consolidations were ill defined, often resembling atelectatic areas. The cultures from these showed Bacillus bronchisepticus and two showed pneumococci. The latter were not agglutinated by pneumococcus type serums nor by the monovalent serum.

SYMPTOMS AND GROSS LESIONS FOLLOWING INTRATRACHEAL INJECTION OF INFLUENZAL MATERIAL

The more marked effects of intratracheal than of intraperitoneal injection were very apparent. The respiratory embarrassment on intratracheal injection, particularly in the infections that terminate fatally, was often marked immediately after injection and extreme the day following. The thorax was often in full expansion, the eyes had a glazed appearance, lacrimation was frequent, the mucous membranes were cyanotic, the breathing was difficult, rapid, irregular, and chiefly abdominal. The animals were restless and irritable, the fur ruffled. Expiratory efforts were often violent, and recurring coughing and choking spells resembling the bronchial spasm of acute anaphylaxis were common. The degree of respiratory embarrassment in the animals that died within twenty-four or forty-eight hours was found to vary considerably during the hours of observation. There were periods of some minutes when breathing, although rapid, was quite free and easy, and the animals often ate food or drank water. The quiescent intervals were followed by a return of marked difficulty in breathing, during which time, bloody, edematous fluid sometimes escaped from the nostrils. Finally the animals, while perfectly conscious, and bending every effort at breathing, would run about aimlessly with the head extended, often jump out of the basket in violent efforts to get breath, and die with symptoms of acute anaphylactic shock, and in addition

with large amounts of hemorrhagic edema fluid escaping from the nostrils. The symptoms in these animals were clearly those of a prolonged anaphylaxis.

The lungs in the animals that died early were always voluminous, dark purplish red, and showed marked hemorrhage and edema with little or no true consolidation. This was true even in those in which the toxicity of the culture killed them in the course of a few hours (figs. 2 and 6), and even following intranasal insufflation. The dark, hemorrhagic and edematous areas often occupied almost the entire lung, but they were always more marked in the posterior lobes. The emphysema was often so extreme that the alveoli were distended to the rupturing point and in some instances rupture was indicated by the finding of subpleural, interstitial emphysema and by the escape of air into the pleural cavity and in the mediastinal and subcutaneous tissues about the chest. The cut surface was extremely wet and large amounts of hemorrhagic edema fluid escaped. The hemorrhagic, edematous areas were often wedge-shaped with the base toward the pleura, or peribronchial. The cyanosis in some of these animals became extreme. The blood was very dark and often remained liquid. In the animals that showed the symptoms described, and that died in two or three days, the lungs were also extremely voluminous and presented the picture of massive pseudolobar pneumonia. At times the consolidation involved almost the entire lung (fig. 3), but although most or all of certain lobes were involved the consolidation was not uniform or complete, but consisted of coalescing areas of lobular pneumonia varying in age and surrounded by areas of hemorrhagic edema. These lungs also contained large amounts of a thin, watery, bloody exudate, and were extremely wet on the cut surface; this was in sharp contrast to the areas of consolidation noted following intratracheal injection of type pneumococci. The smaller bronchi were often found plugged with a bloody exudate, and the mucous membrane of the trachea and larger bronchi was extremely red, and the lumen filled with a blood-tinged froth. These characteristic changes in the lung tended to occur also in the white rat and monkey. A small percentage of the guinea-pigs (about 10%), which showed soon after injection the symptoms of respiratory embarrassment just described, might live for some days with extremely rapid but not difficult breathing, and then die with compressed lungs from hemorrhagic fluid filling the thorax. In these the symptoms of anaphylaxis might be noted at intervals. Usually,

however, death seemed to occur from want of air from a rapidly filling thorax. If death in these animals occurred late, the picture was that of hemorrhagic empyema. The fluid in the pleural cavities, whether death occurred early or late, was almost without exception tinged with blood and contained a relatively small amount of fibrin.

Some animals with not very marked symptoms recovered either in a few days or died at a later period. The symptoms of those that recovered usually consisted of a varying degree of increased respirations, of cyanosis of the mucous membrane with evidence of general illness in loss of action and weight, and in fever. The animals sat humped up and with ruffled hair. The drop in leukocytes lasted for from one to three days. When these animals were anesthetized for examination relatively little lung involvement was found, consisting of irregular areas of partial consolidation, often lobular and peribronchial with hemorrhage and edema, while some showed no lung involvement even when examined within four or five days after injection. The tracheobronchial lymph glands were almost constantly found enlarged and markedly edematous on the cut surface.

The animals that died from three to ten days or more after injection usually showed bronchopneumonia of varying extent associated with emphysema and hemorrhagic edema of various degrees of intensity. In some the pneumonia was lobar in distribution, but lobular in character. Some of the animals developed mucopurulent discharge from the nose associated with maxillary sinusitis and marked redness of the nasal mucous membrane. Occasionally after recovery seemed to be complete there was a return of respiratory embarrassment and death occurred from hemorrhagic edema associated with well defined areas of grayish bronchopneumonia. In not a few of these localized abscesses were noted in the areas showing consolidation.

Cultures from the blood of the animals that died within forty-eight hours were usually positive, but the number of colonies was relatively small, while in those that died later the cultures usually remained sterile. The cultures from the lung and pleural exudates were always positive in the animals that died soon after intratracheal injection, but were often negative in the animals anesthetized while recovering. The relative preponderance of the different strains isolated is shown in table 3. In some animals that died in from ten days to two weeks or more after injection, an entirely different picture supervened. In these the respirations became progressively slower as unconsciousness, great weak-

ness, and a tendency to retraction of the head developed. In a few instances the animals appeared mentally deranged. The lungs were usually small, although occasionally there was moderate emphysema and lesions were slight or wholly absent. The brain and cord were soft, the cerebrospinal fluid was clear but increased in amount; the meninges were edematous and congested. Cultures from the brain and cord substance and spinal fluid were usually negative on blood-agar plates, but in some instances yielded green-producing streptococci in tall tubes of glucose brain broth. The blood in these was always sterile.

MICROSCOPIC ANATOMY OF THE LUNGS

The microscopic findings in the lungs of guinea-pigs varied greatly, depending on the method of injection and on how long after injection the animals survived. In those injected intraperitoneally or subcutaneously the lung findings were relatively slight and consisted of localized hemorrhage and edema with a minimal amount of leukocytic infiltration and desquamation of alveolar epithelium. The localization of the streptococci in the tissues about the capillaries and in the swollen and degenerating alveolar epithelial cells in their normal position or about the desquamated cells which showed nuclear degeneration was a striking picture (figs. 10 and 11). But the lungs of the animals injected intratracheally showed the changes that have come to be regarded as more or less characteristic of influenzal pneumonia. They showed marked distention of alveoli and of alveolar ducts with red blood cells, precipitated serum and a varying number of desquamated degenerating epithelial cells often resembling polymorphonuclear leukocytes, almost complete absence of leukocytes in the acute lesions (figs. 12, 16 and 17), and relatively few leukocytes, even in the more advanced stages of consolidation (figs. 13 and 15a). This picture was in sharp contrast to the consolidation due to type pneumococci (fig. 18a) and the consolidations noted occasionally following injection of the bacteria from normal throats. Besides the marked edema and hemorrhage, probably the most striking change noted in the lungs of these animals was the marked and widely disseminated areas showing necrosis of alveolar epithelial cells and interalveolar capillaries (fig. 19a), also a picture in sharp contrast to that noted following injection of type pneumococci (fig. 19b). The latter finding was strikingly similar to that first noted and so clearly described by LeCount in the case of influenzal pneumonia in man. In the experi-

mental animal in which dosage, place of inoculation, and duration of experiment could be controlled, the cause of this necrosis and the resulting hemorrhage and edema has been found to be due to the localization and growth of the micro-organisms in these structures. The number of organisms was often so large that the outline of alveoli and alveolar ducts could readily be made out with the low power in sections stained by Gram-Weigert by means of the dark lines due to huge numbers of streptococci revealed under higher magnifications (figs. 14c, 15b and 16b). This, too, was in sharp contrast to the even distribution of type pneumococci throughout the highly cellular exudate filling the alveoli (fig. 18b) in experiments in lobar pneumonia in the guinea-pig.

The marked edema and dilatation in the perivascular lymph channels noted in many sections was likewise associated with the presence of enormous numbers of Gram-staining diplococci (fig. 20). Moreover, marked hemorrhagic pleuritis was invariably accompanied by the localization and growth of the micro-organisms in enormous numbers in the subpleural lymphatics (figs. 20, 21 and 22).

It has been possible to study the reparative process of the lungs in animals that were recovering from the effects of injections. The striking feature in the cellular reaction throughout was the relatively small part played by polymorphonuclear leukocytes and the large part played by the proliferated fixed tissue cells, probably endothelial leukocytes and the marked proliferation of epithelial cells.

Distinctive features in the gross and microscopic findings were lacking in the lungs of animals that died soon after injection of the various bacteria, green-producing streptococci, hemolytic streptococci, and staphylococci, except that the hemolytic streptococus tended to invade the pleura and produce hemorrhagic empyema more than the green-producing streptococcus. This is in accord with the findings in the lungs of persons dying from influenzal pneumonia, reported by Blanton and Irons. In the animals that lived for a longer period after injection of mixtures staphylococci were isolated in relatively large numbers, and the sections showed staphylococci in larger numbers or in pure form in the localized areas showing marked leukocytic infiltration, and in abscesses when streptococci were the predominating organisms in the larger intervening areas of hemorrhagic edema showing few leukocytes. The tendency of staphylococci to displace the streptococcal flora in the prolonged experiment even when pure cultures of the streptococci had been injected was often a striking

feature (case 2787, fig. 8). These findings in general are in accord with those in human lungs described by Lord,[13] Weichselbaum,[23] Kuskow,[9] and others in previous epidemics of influenza, and by Le Count,[11, 12] MacCallum,[17] Bell,[2] Chickering and Park,[5] Lucke, Wight and Kime,[15] Opie,[18] Lubarsch,[14] Lyon,[16] and others during the recent epidemic.

Altogether, the virulency more than the species of organism injected determined whether hemorrhagic edema with slight leukocytic infiltration, or bronchopneumonia with marked leukocytic infiltration dominated the picture. As a rule, the leukocytic infiltration in the lung occurred more rapidly and to a greater degree in the animals that showed relatively slight leukopenia or even leukocytosis, and in those injected with cultures from patients with mild attacks who had little or no reduction or even a moderate increase in the leukocyte count than in animals injected with strains from cases showing marked leukopenia.

LESIONS OF THE FEMALE GENERATIVE ORGANS AND OF TISSUES OTHER THAN THOSE OF THE LUNG

By far the most important effects or lesions which have been noted outside of the respiratory tract were those of the female generative organs, especially the uterus, and those of the intestinal tract. A consideration of the latter is reserved for a separate paper.

The effect on the female generative organs in influenzal infection is so marked that many authors regard this as of diagnostic importance. The symptoms most commonly encountered are the occurrence of menstruation for the first time in young girls, of intermenstrual hemorrhages in women in whom the menstrual function has been established, its recurrence after the menopause, and the marked tendency to abortions associated with a high mortality rate in pregnant women.

We have injected, altogether, 98 female guinea-pigs, 76 intratracheally and 22 intraperitoneally or subcutaneously with bacteria from influenza. Of these, 61 died as the result of the injection (62%), and 37 either recovered or were anesthetized as recovery appeared likely, or after being caged with males for several months.

A study of the uterus and the other generative organs was made in 75 guinea-pigs, 57 injected into the trachea and 18 intraperitoneally or subcutaneously. Of the 75, 34 were undoubtedly pregnant at the time of injection as shown by examination after death. Only 6 of these showed normal uteri and normal placental masses when examined.

Four were anesthetized, and 2 died in one and eight days, respectively, from the effects of the injection. The cultures from the uterus in 5 were negative; 1 showed a few green-producing streptococci. In the remaining 28 pregnant guinea-pigs the uterus was either found empty with hemorrhagic areas marking the site of placental attachment or it contained one or more detached or attached hemorrhagic placental masses (fig. 9). Cultures from the hemorrhagic placental masses and bloody mucous in the uterus in these often showed exceedingly large numbers of the bacteria injected.

The uterus of the 27 guinea-pigs that died which were not pregnant showed a varying number of hemorrhagic areas in the endometrium. These were usually small in number and the individual hemorrhages relatively small, but in some instances, even in young guinea-pigs, the hemorrhages were more extensive and occurred over wide areas (fig. 9). They almost always occurred in the horns and rarely in the body of the uterus, cervix, or vagina. Lesions of the latter, however, were noted in the guinea-pigs injected with the streptococcus from the vaginal discharge in case 2809. Marked evidence of infection of the mucous membrane was usually limited to the areas marking placental attachment. In these and in the hemorrhagic placental masses, large numbers of the organisms injected were demonstrable in sections. There was a marked difference between the strains with respect to their power to invade the uterus. In some, all animals injected aborted; in others few or none. The affinity for the uterus was particularly marked in the cultures from the patient (case 3175) who aborted. Intratracheal injections of the sputum in a series of female guinea-pigs and a cat were followed by localization in the uterus and abortion in every animal injected.

The effects in the lung associated with marked bronchial spasm and emphysema, the finding in animals of violent contractions in the uterus immediately after death, and in some instances when anesthetized, and the absence of demonstrable infection either in the placental site or the mucous membrane of the uterus in some of the animals injected, are good reasons for the belief that the emptying of the uterus may be due in some instances to the violent contractions of the uterus from the formation and circulation of "anaphylatoxin" and may not always be the result of actual localization of the bacteria at the placental side.

Lesions of the ovary were relatively rare. In some instances, however, one or both were edematous, fully twice the normal size, and in sections evidence of degeneration of cells in the granular layer associated with edema and leukocytic infiltration in the graafian follicles were noted. Lesions in the interstitial tissues of the ovary were not found.

Cultures were made from the sections in the uterine horns in 75 guinea-pigs. In these the amount of material cultivated usually consisted of only one or two drops from the ends of a small pipet. Green-producing streptococci in varying numbers were isolated in pure culture or together with hemolytic streptococci and staphylococci in 28 animals, hemolytic streptococci in 10, and staphylococci in 25.

Altogether, 10 female guinea-pigs were injected with the control cultures, including those from normal throats, from cases of simple nasopharyngitis, and with type pneumococci from lobar pneumonia. Of these 10 were pregnant. Only 3 showed slight lesions of the uterus or placental masses and only 1 aborted. Cultures were made from the uterus in 10. One showed a few colonies of staphylococci; the rest remained sterile. It is thus apparent that the marked affinity for the uterus and the high incidence of abortions in the animals injected with the influenzal strains is not shared by the control strains. When we were dealing with controlled conditions the effects on the female generative organs in the guinea-pig paralleled in so far as is possible those observed in women.

In order to determine whether other effects on the female generative organs might not have occurred following injection of these strains, the female guinea-pigs that survived were mated with males and kept under observation for from two to three months. Only 2 became pregnant, 1 showing 1, the other 2 fetuses. The general health of all these animals appeared to be good. They gained in weight. There was no evidence of disease of the external generative organs either in the males or females and no gross lesions of ovaries, uterus, or vagina in the animals chloroformed to determine the presence or absence of pregnancy. It would appear, therefore, that infection with these micro-organisms had a pronounced depressant effect on the female generative mechanism after a recovery in other respects seemed to be complete. On the basis of this experiment a diminution in the birth rate in human beings greater than can be accounted for by the death of women of the child-bearing age might be expected.

The kidneys often showed a marked degree of diffuse, cloudy swelling and less commonly, focal areas of infection situated most often in the medulla; in a few instances these areas seemed to have given rise to pyelitis. Hemorrhages in the mucous membrane of the bladder were rarely noted. The suprarenals were often much swollen and hemorrhagic on the cut surface.

Following intratracheal injection of a few strains, numerous lesions of the muscles occurred, and in a few animals single large, hemorrhagic, edematous, necrotic areas were noted in the abdominal rectus muscle. Lesions of the myocardium occurred not infrequently and consisted usually of a grayish white diffuse degeneration. The ventricles in most of these were of stony hardness and in firm systole.

Lesions of the stomach were rare and occurred almost exclusively in animals that died from overwhelming infection, and consisted almost wholly of small localized hemorrhages with or without superficial ulceration associated with marked distention of the stomach with gas rich in carbon dioxid and marked postmortem digestion of the stomach wall.

General peritonitis following intratracheal injection was noted in 12 guinea-pigs. This occurred usually only when marked pleuritis or empyema was present or when it was otherwise secondary to infection of the uterus and tubes.

EXPERIMENTS ON THE MECHANISM OF RESPIRATORY EMBARRASSMENT IN INFLUENZA

Many findings in influenzal pneumonia, and particularly those in guinea-pigs following injection of bacteria from influenza, suggest strongly that they may be due in part to the formation of "anaphylatoxin," and that the lung picture may be the result of a prolonged anaphylaxis, associated with bronchial spasm. The protective effects of epinephrin and atropin against fatal anaphylactic shock are thoroughly established. It was thought, therefore, that injections of these substances into guinea-pigs having symptoms resembling anaphylaxis might furnish experimental evidence of the nature of the respiratory embarrassment and the use of these substances in treatment.

The effects of subcutaneous injection of epinephrin were studied in 15 guinea-pigs. The dose ranged from 0.02 c c-0.05 c c of a 1:1,000 solution of epinephrin chlorid to each 100 gm. of body weight. Good effects were noted in all but 3 guinea-pigs which showed no improve-

ment; respiratory embarrassment was found to be due either to filling of the pleura or to extensive consolidation of the lung. The improvement, although striking, was always temporary, lasting from one-half to five hours. In a few control experiments in which the same dose of culture was given, life appeared to be prolonged for from one to two days in the animals treated with epinephrin, but in no instances in which recovery took place could it be attributed to the effects of this drug. This would be expected because of the inexhaustible supply of anaphylatoxin causing bronchial spasm due to the multiplication of the bacteria. Protocols illustrate the results obtained.

Guinea-pig 750, weighing 350 gm., was injected intraperitoneally Nov. 25, 1918, at 5:30 p. m. with 0.3 c c of the sputum from case 2620. The white blood count before injection was 14,400. November 26 at 9 a. m. the animal appeared to be sick, the fur was rough, and the respirations were rapid and difficult. The chest appeared to be dilated, and breathing was accomplished chiefly by means of the diaphragm. There was an expiratory rattle in the throat, and the animal's repeated forced efforts at expiration resembled the symptoms of anaphylactic shock. The mucous membrane of the conjunctiva, mouth, and tongue was blue. The leukocyte count was 4,500. At 1 p. m. the condition was unchanged except that respiratory efforts were more labored. A small amount of fluid oozed from the mouth and there was intense cyanosis of the mucous membranes. At 2:30 p. m. the condition was about the same. At this time 0.2 c c of a 1:1,000 solution of epinephrin chlorid were injected subcutaneously. At 2:35 p. m. there was no apparent change in respiration. At 2:45 p. m. the respirations undoubtedly were less labored and the animal appeared to be improved. At 3 p. m. the respirations appeared to be quite normal and the animal appeared to be much improved. At 3:45 p. m. the animal seemed to be comfortable, ate food, and the respirations were only slightly above the normal; cyanosis was absent. At 5:30 p. m. the respiratory difficulty had returned to some extent and the animal had a violent attack resembling anaphylactic shock. November 27, at 8:20 a. m., it was found dead. It showed hemorrhagic serofibrinous peritonitis and moderate emphysema of the lungs (12 c c), and beginning bronchopneumonia associated with marked edema surrounding the consolidated areas. The blood was very dark and had not coagulated. Blood-agar plates of the blood showed a moderate number of green-producing streptococci; from the peritoneal fluid there was a large number in pure culture.

Guinea-pig 965, weighing 360 gm., was injected intratracheally Jan. 14, 1919, at 3 p. m. with 2 c c of the glucose-brain-broth culture of the vaginal swab of case 2809. At 7 p. m. the respirations were extremely rapid and chiefly abdominal, the chest was dilated, the hair ruffled; there was an expiratory grunt, and the animal was restless, appeared uncomfortable, coughed repeatedly, and scratched its nose at intervals; bloody, edematous fluid escaped from the nostrils. At 7:20 p. m. respiratory embarrassment was unchanged. At 7:30 p. m., 0.2 c c of a 1:1,000 solution of epinephrin chlorid were injected subcutaneously. At 7:45 p. m. the picture had completely changed. The respirations were free and easy; the animal walked about, and the discharge of bloody fluid from the nose had ceased. At 11:15 p. m. the respirations were growing more labored; the animal was weak and restless, and breathing was difficult;

there was an expiratory rattle, and the bloody discharge from the nose had returned. At 11:30 p. m. the animal had an attack of severe shortness of breath in which it made violent efforts to breathe, ran around its mate, jumped into the air in a last violent effort at breathing, fell on its side as bloody fluid spurted from the nose and mouth, and died. The lungs were voluminous (22 c c); practically the entire lung was hemorrhagic and filled with hemorrhagic edematous fluid. The peribronchial lymph glands were edematous. A small amount of bloody fluid was found in the pleural cavity and a large subcapsular hemorrhage in the lower pole of the left kidney. Sections of the lung showed marked dilatation of alveoli filled by hemorrhagic edematous fluid, with slight leukocytic infiltration, and large numbers of diplococci in the hemorrhagic areas (figs. 17 a and b).

RELATION OF MORTALITY IN GUINEA-PIGS TO VIRULENCY OF THE ORGANISM ISOLATED IN FATAL AND NONFATAL INFLUENZA IN PATIENTS

If the results obtained in the animals really indicate close etiologic relationship of these streptococci to the disease, the relative mortality in the animals should correspond roughly with that in the patients from whom the material for injection was obtained. During the course of the experiments, the impression was gained that the material from severe or fatal cases is more virulent, producing more severe respiratory embarrassment, more marked hemorrhagic edema, and a higher mortality rate than the material from patients with mild attacks who recovered. It was considered of value, therefore, to determine the mortality in the animals according to whether the material injected was from patients with influenza and influenzal pneumonia who recovered, or from patients with influenza and influenzal pneumonia who died. In table 4 is given the mortality according to the diagnosis made at the time the material injected into animals was obtained and according to whether the patient died or recovered.

The mortality in the animals injected (intratracheally and intraperitoneally) with material from patients with influenza in whom signs of lung involvement were slight or entirely absent at the time of the experiments and who recovered, was about the same (49%) as in patients with influenzal pneumonia who recovered (43%). The 3 persons who had influenza at the time of the experiments and who later died of influenzal pneumonia harbored streptococci which killed the 5 guinea-pigs injected. It should be noted that the mortality in the guinea-pigs injected with material from patients with influenzal pneumonia was 26% lower when the material was taken from patients who recovered than when taken from those who died. In the former it

was 43% in 23 guinea-pigs injected with 23 strains; in the latter, 69% in 78 guinea-pigs injected with 39 strains. The average mortality following intratracheal injection of 67 strains in 109 guinea-pigs was

TABLE 4

MORTALITY IN GUINEA-PIGS ACCORDING TO PLACE OF INJECTION AND MATERIAL INJECTED, AND ACCORDING TO DIAGNOSIS AND ULTIMATE RESULT IN PATIENTS FROM WHOM MATERIAL WAS OBTAINED

Place of Injection and Material Injected	Diagnosis at Time of Animal Experiments and Ultimate Result	Number of Strains	Number of Animals			Percentage of Mortality
			Injected	Recovered	Died	
Trachea—sputum, primary culture, green-producing streptococci, hemolytic streptococci, staphylococci	Influenza—recovery	32	43	23	21	49
	Influenzal pneumonia—recovery	12	11	7	4	36
	Total	44	55	30	25	[45]
	Influenza—death	1	3	0	3	100
	Influenzal pneumonia—death	22	51	20	31	61
	Total	23	54	20	34	[63]
	Total all strains	67	109	50	59	54
Peritoneum—sputum, primary culture, green-producing streptococci, hemolytic streptococci, staphylococci	Influenza—recovery	36	42	21	21	50
	Influenzal pneumonia—recovery	11	12	6	6	50
	Total	47	54	27	27	[50]
	Influenza—death	2	2	0	2	100
	Influenzal pneumonia—death	17	27	4	23	85
	Total	19	29	4	25	[86]
	Total all strains	66	83	31	52	63
Trachea and peritoneum—sputum, primary culture, green-producing streptococci, hemolytic streptococci, staphylococci	Influenza—recovery	68	86	44	42	49
	Influenzal pneumonia—recovery	23	23	13	10	43
	Total	91	109	57	52	[48]
	Influenza—death	3	5	0	5	100
	Influenzal pneumonia—death	39	78	24	54	69
	Total	42	83	24	59	[71]
	Total all strains	133	192	81	111	58
Vein—primary culture, green-producing streptococci, hemolytic streptococci	Total all strains	9	19	5	14	74
	Grand total (111 cases)	142	211	86	125	59

54%. The average mortality in the 55 animals injected with 44 strains from patients who recovered was 45% in contrast to a mortality of 63% in the 54 guinea-pigs injected with 23 strains from patients who died. The results following intraperitoneal injection were similar.

The average mortality in 83 guinea-pigs injected with 66 strains was only 9% higher, 63%, than following intratracheal injection. The average mortality in 54 guinea-pigs injected with 47 strains from patients who recovered was 50%, in contrast to a mortality of 86% in the 29 guinea-pigs injected with 19 strains from patients who died. A summary of the results following these two methods of injection gives a total average mortality of 58% in 192 guinea-pigs injected with 133 strains from 111 cases, the average mortality in 109 guinea-pigs injected with 91 strains from patients who recovered being 48% in contrast to the mortality of 71% of the 83 guinea-pigs injected with 42 strains from patients who died. The average mortality following intravenous injection of 19 guinea-pigs with 9 strains was 74%. The grand total average mortality in 211 guinea-pigs injected with 142 strains, derived from 111 cases, was 59%.

It would seem from these facts that the virulency of the streptococci in patients who recover is less marked than in patients who die.

GENERAL DISCUSSION AND SUMMARY

The animal experiments that have been carried out heretofore with bacteria isolated quite constantly in influenza, both in 1889 and 1918, have consisted largely of virulency and toxicity tests in which only the usual methods of injection were used, and in which sufficient attention was not directed to the time and method of cultivation before injection. Statements have appeared concerning the high virulency of organisms of the streptococcus group [3, 7] and the ability of influenza bacilli to produce highly toxic products in cultures [19] and a tendency to produce lesions in the lung,[1, 8] but little has been accomplished in the way of reproducing the clinical and pathologic picture of epidemic influenza.

Intratracheal injection has been employed only occasionally in previous studies despite the fact that by this manner of injection of highly virulent pneumococci Lamar and Meltzer [10] have produced the typical picture of lobar pneumonia in the dog, Winternitz and Hirschfelder [24] in the rabbit, Cecil and Blake [4] in the monkey, and Wollstein and Meltzer [25] produced bronchopneumonia in the dog with bacteria isolated from bronchopneumonia in man.

The results of subcutaneous, intraperitoneal and intravenous injections, in my hands, show that the bacteria, particularly green-producing streptococci, isolated quite constantly in epidemic influenza possess

high and peculiarly invasive powers. They have a marked tendency to produce leukopenia, to localize electively in the interstitial tissues and epithelial cells of the alveoli and smaller bronchi, and to produce hemorrhage and edema in the lungs, as symptoms of anaphylaxis and emphysema of the lung usually develop. Significant as these facts are, an accurate analysis of the effects of the bacteria and the precise rôle they play in influenza was possible only by the use of methods which simulated more closely the natural conditions, through the application of the bacteria to the normal uninjured epithelium of the lower respiratory tract by the method of intratracheal injection. The guinea-pig was considered the most suitable animal available for this study. Its resistance to streptococcal infection, although higher than that of man, its reaction to bacterial poisons and anaphylaxis are in general quite similar.

There has been much discussion, based chiefly on the results of cultures, with regard to the relative importance of the four main types of bacteria isolated in this disease, green-producing streptococci, including pneumococci, hemolytic streptococci, staphylococci and influenza bacilli. By a combined study of intraperitoneal injection in mice and guinea-pigs and intratracheal injection in guinea-pigs of sputum and lung exudates directly, and of the primary mixed culture of standard dosage a fair knowledge of the degree of the invasive power of these bacteria has been obtained. Invasion by the green-producing streptococcus in pure or almost pure form occurred in most instances even when the bacteria were not present in predominating numbers in the material injected. In some instances invasion by hemolytic streptococci occurred, but usually only when they were present alone or in predominating numbers, and more rarely by staphylococci, but only when they were present in predominating numbers in the material injected. Invasion by influenza bacilli following injection of sputum or lung exudate, which in some instances was proved to contain influenza bacilli, has not occurred in a single experiment. Similar results regarding the relative invasive power of these species have been obtained by injections of pure cultures of each, and again the independent invasive power of influenza bacilli was found to be slight. It should be emphasized that while it was necessary to use rather large doses for routine injections, owing to the relatively high and variable resistance of guinea-pigs and marked variations in invasive powers of the strains, small numbers of the more virulent streptococci sufficed to

produce characteristic lesions. They followed intratracheal application of filtrates, intranasal insufflation of particularly virulent cultures and in a few instances through contact infection from guinea-pigs injected with especially virulent cultures.

The effects of intratracheal injection of mixtures of these organisms as they occurred in sputum and primary cultures and of pure cultures of recently isolated strains, varied within wide limits. The animals may be classified in four groups as follows:

Group 1. Animals that showed slight symptoms and then recovered.

Group 2. Animals that showed mild early symptoms and later suffered severe attacks.

Group 3. Animals that showed severe and progressive symptoms of marked lung involvement.

Group 4. Animals that showed extreme and rapidly fatal effects.

Group 1.—The symptoms of the animals in this group were relatively slight, consisting in the main of moderate illness, loss in weight, usually some fever, moderate leukopenia, and slight or moderately increased respirations for a number of days, followed by complete recovery. The animals were found to be immune to subsequent injections of heterologous strains. Those anesthetized for examination showed relatively slight or no lung involvement; the blood was sterile and the lungs were either sterile or contained a few of the organisms injected. These findings may be considered to parallel the clinical findings in patients with relatively mild influenza in whom little or no lung involvement can be demonstrated and in whom relative immunity is conferred as in the animals.

Group 2.—In the animals in this group the initial effects of the injection were more pronounced and lasted longer than those in the animals of group 1. Some of the animals, after apparent recovery, developed severe symptoms of respiratory involvement and died in from one to two days with anaphylactic symptoms, voluminous lungs, and hemorrhagic bronchopneumonia or, more rarely, from hemorrhagic pleuritis. In others the symptoms of respiratory embarrassment progressed more slowly; many of the animals developed rhinitis, and later died from purulent bronchitis and well-defined bronchopneumonia, often with small abscesses, and more rarely from abscess and gangrene, or from emphysema with or without bronchopneumonia. The cultures

from the animals that died of relatively acute symptoms in the pneumonic attack usually showed green-producing streptococci, the pneumonic lung showing localized abscesses or abscess, usually staphylococci, or staphylococci and streptococci, and the empyemas usually hemolytic streptococci with or without staphylococci. The findings in this group may be regarded as representative of the findings in the group of patients with the more severe influenzal attacks who later develop influenzal pneumonia or, more rarely, well-defined coalescing bronchopneumonia with slight hemorrhagic edema, but with purulent bronchitis and localized abscesses or a single large abscess, or of empyema with or without bronchopneumonia.

Group 3.—In this group the initial symptoms were severe and usually progressed without intermission until death occurred in from two or three days from an increasing intense cyanosis and respiratory rate or from marked respiratory embarrassment from anaphylactoid symptoms during which in many instances, hemorrhagic edematous fluid escaped from the nose while the animals made violent efforts to breathe. The lungs were huge, in a few cases interstitial emphysema had occurred and extensive consolidation consisting of coalescing areas of pneumonia of different ages and intervening areas of hemorrhagic edema. The blood was dark and remained liquid for a long time. The postmortem and microscopic findings in this group were in every way like those described as typical of acute influenzal pneumonia in man.

Group 4.—In this group extreme dyspnea often occurred almost immediately after injection of highly virulent cultures and their filtrates. The symptoms were quite typical of acute anaphylaxis and many of the animals died while making violent efforts to breathe, as a bloody fluid ran from the nose and mouth. The lungs were huge, a dark purplish red, and hemorrhagic and edematous throughout. The symptoms and postmortem findings resembled very closely those noted in patients who died soon after being taken ill, usually in the initial attack of acute hemorrhagic edema of the lungs before sufficient time had elapsed for the development of extensive consolidation.

The distress from lack of oxygen, the intense cyanosis, and the extreme efforts at respiration of many animals in the latter two groups resembled the picture presented by patients dying from a rapidly filling lung who copiously expectorated a serous, bloody, frothy fluid, and who frequently sat up or left their beds in making violent efforts to breathe as death occurred.

The experiments with the filtrates show that under the proper conditions green-producing streptococci may become sufficiently small to pass through bacterial filters which prevent the passage of Bacillus prodigiosus, and that small numbers of the streptococci, when applied to the normal mucous membrane of the lower respiratory tract, are sufficient to produce the characteristic symptoms and pathologic changes in the lungs of guinea-pigs. Moreover, the results of the experiments with filtrates of cultures, pneumonic lungs, and sputum, and those on the mechanism of respiratory involvement show that the strains from influenza which have high invasive powers also have the power to produce anaphylatoxin in large amount, as measured by intratracheal injection. Many findings in influnza, such as the expanded, hyperresonant, relatively immobile thorax, cyanosis, the sharp leukopenia, the delayed coagulability of the blood, and the voluminous lung appear to be expressions of an anaphylactoid intoxication.

The results obtained following injection of guinea-pigs with influenzal material were so definite and so striking as to rule out quite effectively the possibility of spontaneous infection. However, this possibility was considered throughout the series of experiments. Only vigorous healthy looking pigs were used. No epidemic of pneumonia occurred among the reserve supply. The patchy areas of chronic bronchopneumonia, usually situated in the anterior lobes noted in guinea-pigs, at times were easily differentiated from the acute lesions due to the injections by their appearance and by the fact that cultures in the former condition nearly always showed Bacillus bronchisepticus: Control injections of salt solution and broth were without effect, and finally similar results followed intratracheal injections of influenzal material in other species (rat, rabbit, cat and monkey).

The effects following injection of the control strains of green-producing streptococci, hemolytic streptococci, staphylococci, and type pneumococci in like dosage were quite different. The immediate symptoms were less marked or absent, the mortality rate was much lower, leukopenia rarely occurred, leukocytosis was the rule, respirations while rapid in some instances, were usually free and easy, prolonged anaphylactoid symptoms did not occur, and hemorrhages from nostrils were not observed. The lungs were smaller, the exudate more cellular, the areas of consolidation occurred earlier and were more definitely outlined, and there was either no edema or relatively slight hemorrhagic edema at all times; marked necrosis of alveolar capillaries and epi-

thelium were also absent. The contrast between the gross and microscopic picture of the lung following injections of highly virulent green-producing streptococci from influenza and of type pneumococci was particularly striking. In the former there was huge dilatation of the lung and alveoli, marked desquamation and degeneration of alveolar epithelium, necrosis of alveolar capillaries associated with peripherally placed streptococci in large numbers, and hemorrhage and edema everywhere with relatively slight leukocytic infiltration. In the case of type pneumococci the striking findings were moderate distention of the lung and alveoli with slight degeneration of epithelium and little change in interalveolar capillaries, but with marked diffuse, sharply demarkated, highly cellular exudate filling the alveoli, with the pneumococci diffusely distributed in the exudate, and with little edema.

The occurrence of marked lesions of the lungs, including well-marked pneumonia following injection of pure cultures of staphylococci and the presence of staphylococci in areas of softening in lungs injected with mixtures, and in large numbers in the sputum in some cases, but more particularly in the lung exudate after death, are in accord with the findings of Chickering and Park in Staphylococcus aureus pneumonia, and emphasize anew the importance of the staphylococcus as a cause of death and a factor in the production of lesions in the lung in epidemic influenza.

The theory that influenza and influenzal pneumonia are manifestations of the same infection varying only in degree is supported by these experiments. The bacteriology of the sputum and other exudates in influenza and of the early stages in influenzal pneumonia have been found to be identical. The infecting powers of the strains isolated in these two conditions, particularly of the green-producing streptococci, have been found to be very similar. The mortality in the guinea-pigs injected with strains from influenza is as high as in those injected with strains from patients with influenzal pneumonia who recovered. The mortality in the guinea-pigs was proportionately higher in those injected with material from patients who died than in those injected with material from patients who recovered. The leukocyte curves in the fatal and nonfatal infections in the guinea-pig ran parallel with the leukocyte curves in fatal and nonfatal infections in persos.

From a study of 266 cases of influenza and influenzal pneumonia in which accurate record of the exact onset of the attack was obtainable it was found that 145 patients either had no preceding influenzal

attack or developed outspoken signs of pneumonia within three days from the onset of symptoms; 108 became ill with pneumonia after an interval of from four to nine days, and 13 only had an interval ranging from ten to twenty-one days. The number of patients who developed outspoken signs of lung involvement in the initial attack and without a quiescent interval is therefore large, and in general similar to that noted by others. By means of the more refined methods of examination, such as the roentgen ray, the incidence of lung findings in the primary influenzal attack has been greatly increased. Indeed, the manifestations of the disease and the bacteriologic findings in some instances have led good clinicians to regard the so-called complications as the disease itself,[6] and bacteriologists to look on the "secondary invaders" as the cause of sharp outbreaks.[7]

Through a painstaking study of the infecting powers of the streptococci in influenza and influenzal pneumonia throughout several epidemic waves, it has been possible to reproduce in animals, by various methods of injection, but particularly by intratracheal injection, the picture of influenza as seen in man. The symptoms both of influenza and influenzal pneumonia have been closely simulated in these animals as far as possible. Likewise, the gross and microscopic changes which have come to be regarded as quite characteristic of influenzal infection have been reproduced. The same varied picture that often supervenes in the latter stages of influenzal pneumonia in man, such as leukocytosis as evidence of pleural involvement and purulent infection, becomes manifest and the varied pathologic picture in the lung of patients who died late have been noted in guinea-pigs injected intratracheally with these strains. The tendency to involvement of the female generative organs, with a high mortality in pregnancy and a high incidence of abortion, of lesions of the heart, abscess in the rectus muscle, and interstitial emphysema have been noted in the experimental animal quite as they occur in man.

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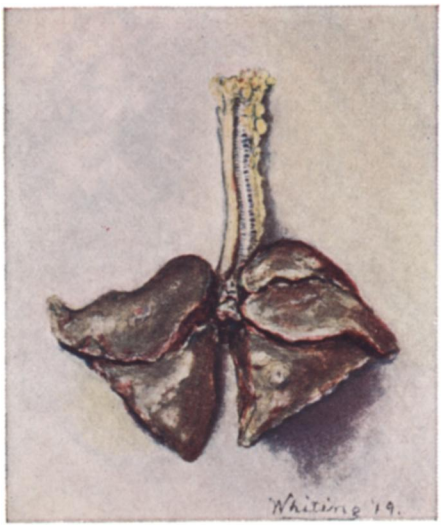
Plate I



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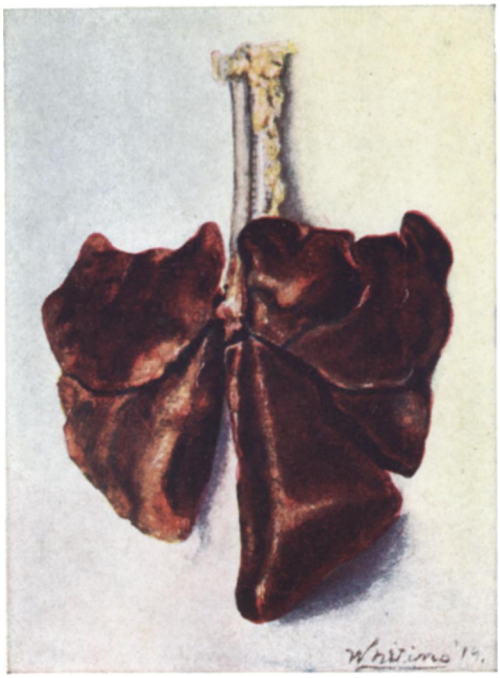

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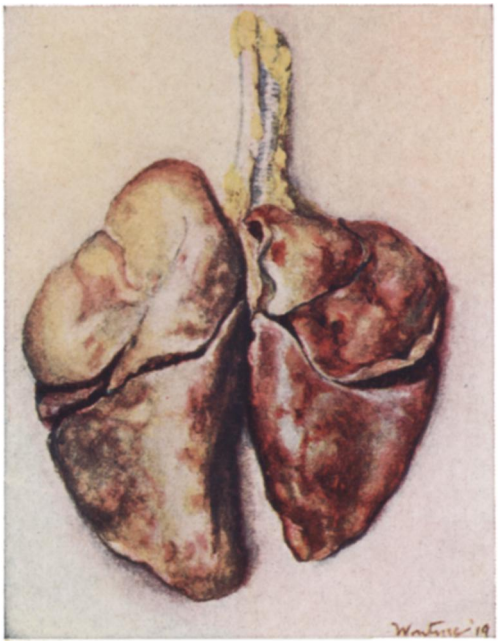

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Plate II

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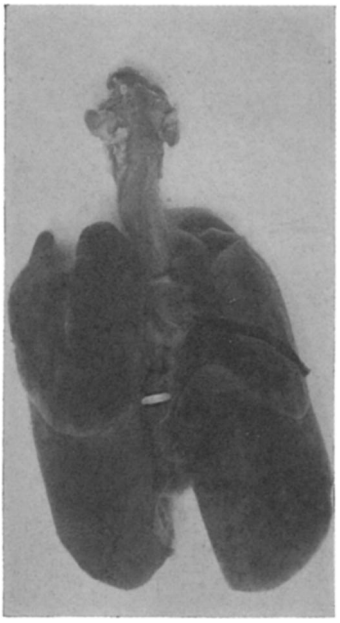

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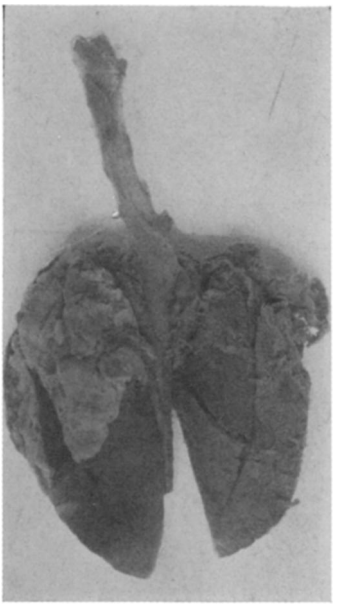

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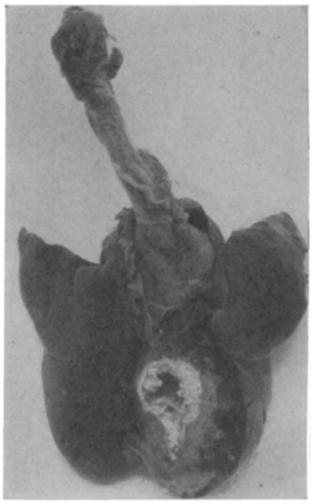

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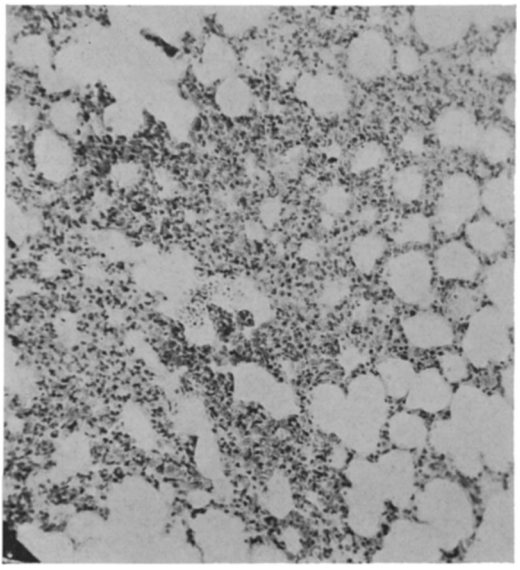
PLATE III



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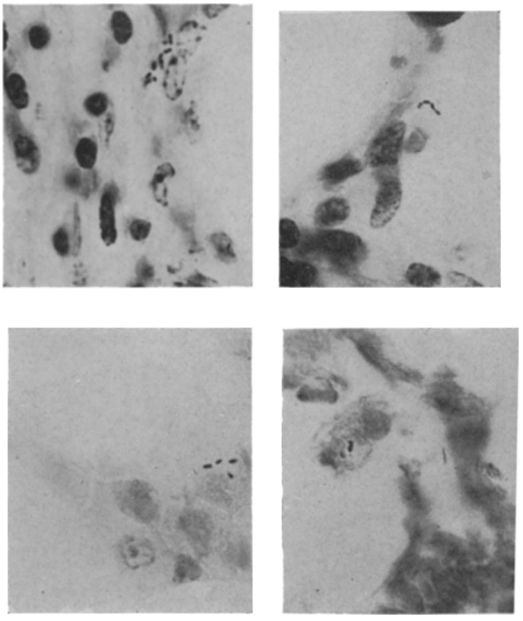

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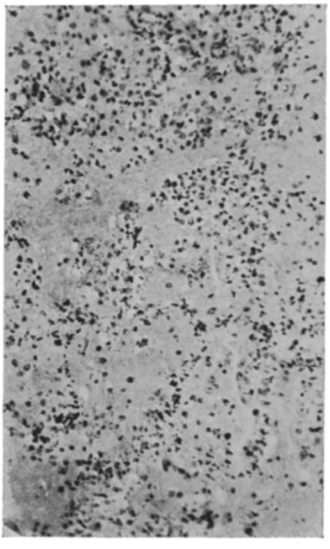

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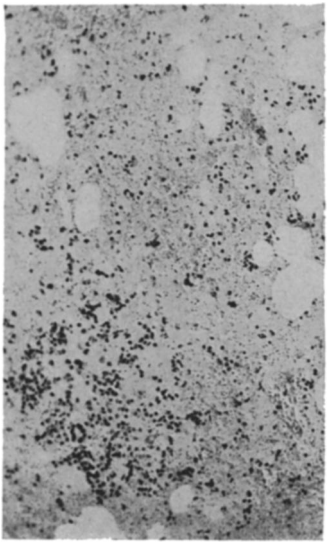

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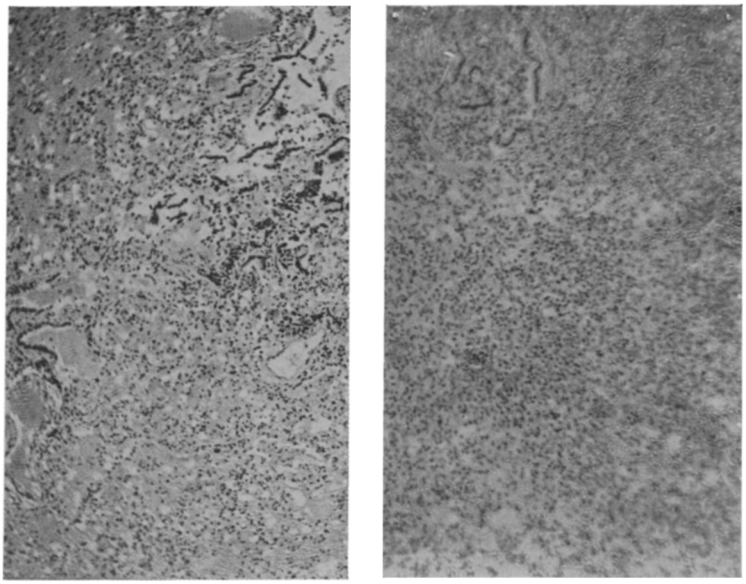

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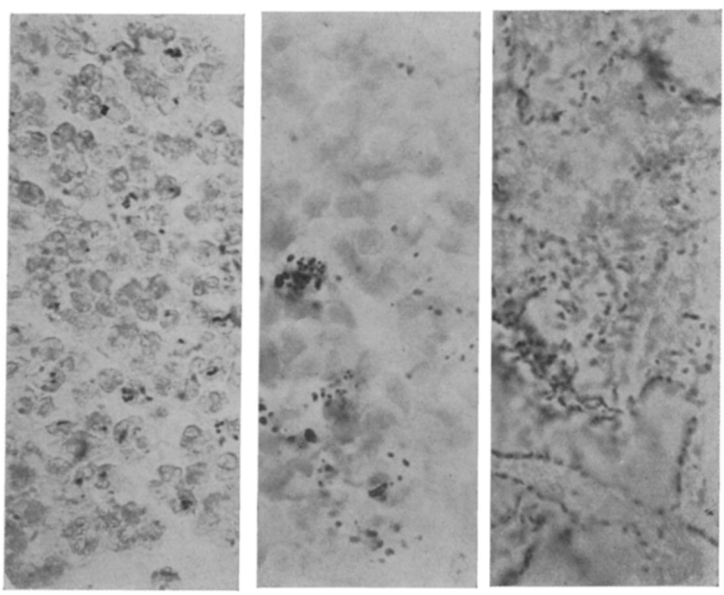

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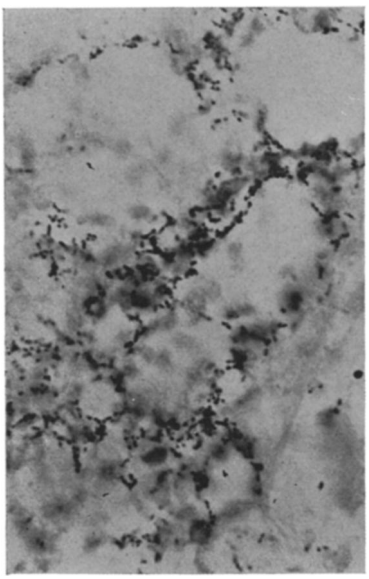

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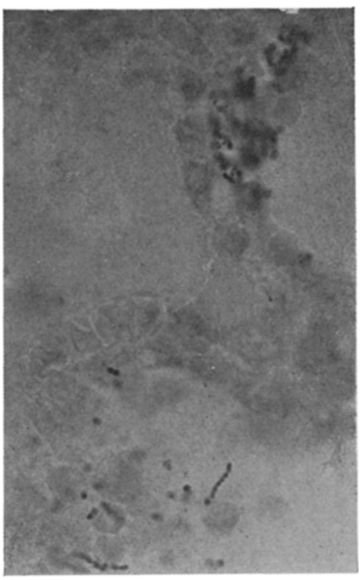

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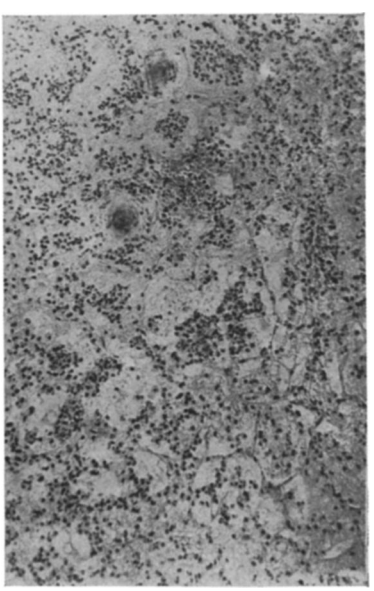

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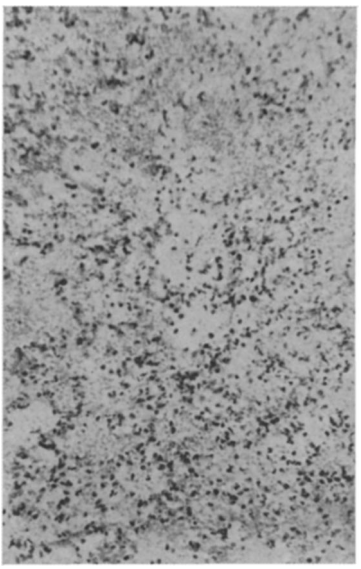

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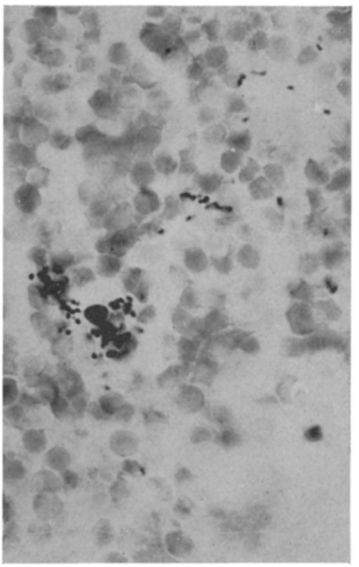

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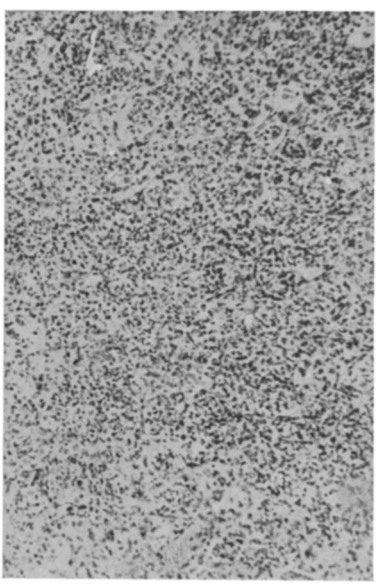

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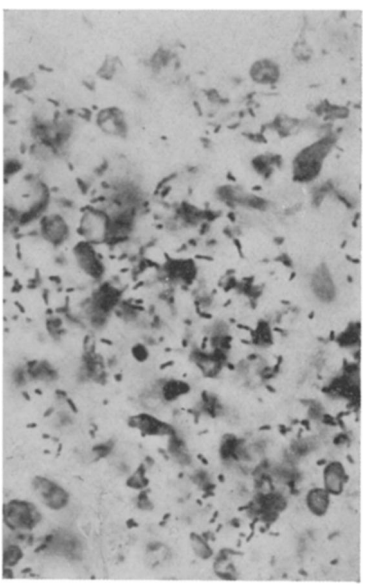

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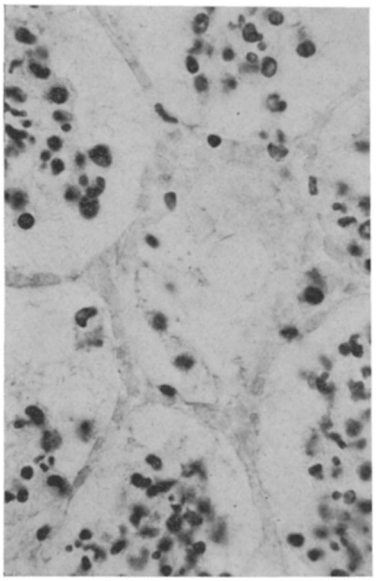

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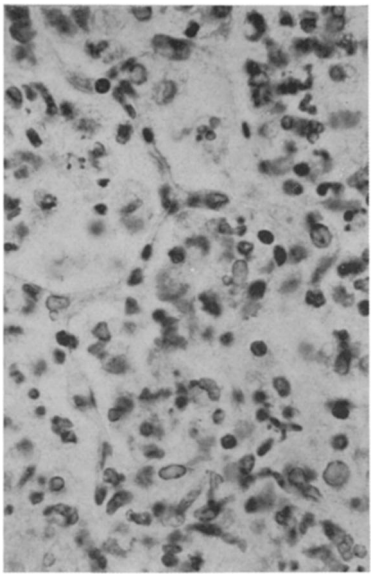

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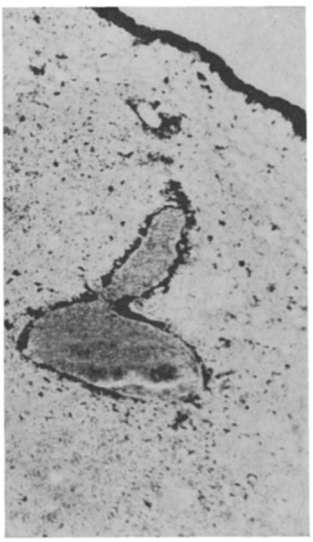

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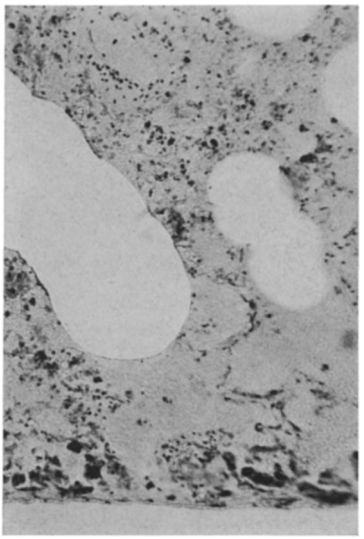

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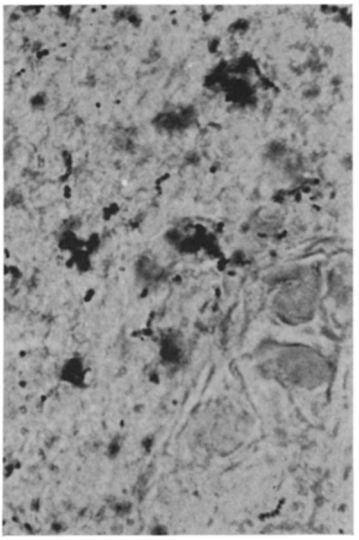

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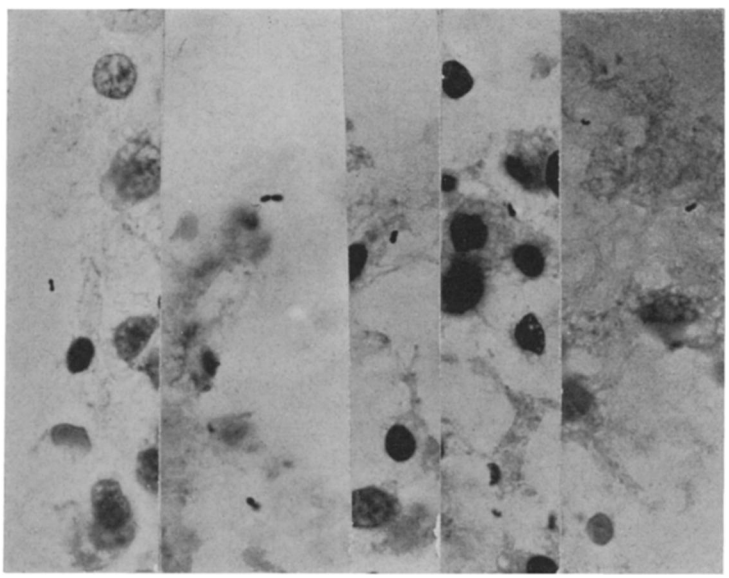

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Explanation of Plates

Plate 1

Fig. 1.—Lung of normal guinea-pig weighing 350 gm., killed with ether. Total volume of lung 5.5 c c, weight 3.5 gm. (× 1).

Fig. 2.—Lung of guinea-pig 1345, weighing 400 gm., showing acute hemorrhagic edema 2½ hours after intratracheal injection of a culture of green-producing streptococcus from influenza in the fourth culture generation. Total volume of lung 23 c c, weight 18 gm. (× 1).

Fig. 3.—Lung of guinea-pig 1249, weighing 380 gm., showing massive pseudolobar pneumonia 48 hours after intratracheal injecton of the primary culture of green-producing streptococcus from the blood of a fatal case of influenzal pneumonia (case 3171). Total volume of lung 26 c c, weight 21 gm. (× 1).

Fig. 4.—Lung of guinea-pig 1448, showing lobar pneumonia 48 hours after intratracheal injection of type II pneumococcus; total volume of lung 14 c c, weight 12 gm. (× 1).

Plate 2

Fig. 5.—Lung of guinea-pig 737 that died 24 hours after intraperitoneal injection of sputum from case 2607. Note the large size (12 c c) compared with the lung shown in figure 1, and the hemorrhage and edema over the posterior aspect (× 1¼).

Fig. 6.—Lung of guinea-pig 1335 injected intratracheally with culture from sputum (case 2623) 40 minutes before death occurred with symptoms of acute anaphylaxis. Total volume of lung 20 c c. Note the extreme hemorrhage and edema (× 1).

Fig. 7.—Lung of guinea-pig 957 injected 6 days previously with hemolytic streptococci from case 2798. Note the marked thickening of the pleura (× 1).

Fig. 8.—Lung of guinea-pig 944, injected two weeks previously with hemolytic streptococci from case 2787, showing a large gangrenous abscess, numerous small abscesses in the cut surface of the right diaphragmatic lobe and marked pericarditis (× 1).

Fig. 9.—Photograph of uteri of three guinea-pigs, laid open presenting anterior view, illustrating the type of lesions noted in this organ following injection of influenzal material. Note the hemorrhages in the mucous membrane of the left horn of the uterus of guinea-pig 861 and both horns of guinea-pig 990, and their absence in the cervix and vagina, the hemorrhagic fetal masses and the localized edema, and infiltration of the endometrium marking placental attachments in guinea-pig 940 (× 1).

Plate 3

Fig. 10.—Section of the lung of guinea-pig 737, injected intraperitoneally with the sputum of case 2607. Note the marked dilatation of alveoli, congestion of the capllaries, and the alveolar and interstitial edema and hemorrhage. Hematoxylin and eosin (× 100).

Fig. 11.—Sections of the lung of guinea-pig 737, shown in figures 5 and 10; (a) diplococci beneath the epithelial cells in the alveolar wall and just outside of a capillary; (b) chain of diplococci in alveolar wall where epithelial cells have desquamated; (c) diplococci in an epithelial cell in its normal position, but showing disintegration of the nucleus in the wall of an alveolus with hemorrhage; (d) diplococci in desquamating epithelial cell. Gram-Weigert (× 1000).

Fig. 12.—(a) Section of lung of case 2800 showing marked hemorrhagic edema; (b) lung of guinea-pig shown in figure 2 with dilatation of alveoli, marked hemorrhagic edema, and dissolution of parenchymatous cells. Hematoxylin and eosin (× 100).

Plate 4

Fig. 13.—Section of lungs showing (a) hemorrhagic edema with relatively slight cellular infiltration and marked destruction and desquamation of the bronchial epithelium in case 2800, and (b) in guinea-pig 956 twenty-four hours after intratracheal injection of the culture of green-producing streptococcus from a single colony from the throat in this case. Hematoxylin and eosin (× 100).

Fig. 14.—(a) Diplococci in the lung of case 2800 shown in figures 12 and 13; (b) diplococci in the lung of guinea-pig shown in figure 12; (c) diplococci distributed along the alveolar lining of the alveoli in the lung shown in figure 13b.

Plate 5

Fig. 15.—Lung of guinea-pig shown in figure 3; marked hemorrhagic edema, dilatation of the alveoli, desquamation and disintegration of the alveolar epithelium, necrosis of capillary epithelium with relatively slight leukocytic infiltration, and many diplococci lining the alveolar walls; (a) hematoxylin and eosin (× 100); (b) Gram-Weigert (× 800).

Fig. 16.—Lung of monkey 228 injected intratracheally with the emulsion of the hemorrhagic mucous membrane of the stomach in case 2979. Note the hemorrhagic edema, desquamation of the epithelial cells of the alveoli and ductus alveolaris, and the diplococci chiefly along the alveolar lining; (a) hematoxylin and eosin (× 100); (b) Gram-Weigert (× 1000).

Plate 6

Fig. 17.—Section of the lung of guinea-pig 965 injected intratracheally with a culture from the vaginal swab in case 2809. Note the dilatation of the alveoli, the marked edema and hemorrhage, the relatively slight cellular infiltration, and the large number of diplococci in the hemorrhagic and edematous areas; (a) hematoxylin and eosin (× 1000); (b) Gram-Weigert (× 1000).

Fig. 18.—Section of consolidated right diaphragmatic lobe of lung shown in figure 4. Note the marked and uniform cellular, chiefly leukocytic infiltration, relatively slight edema, and the even distribution throughout the alveolar exudate of the pneumococci; (a) hematoxylin and eosin (× 100); (b) Gram-Weigert (× 1000).

Plate 7

Fig. 19. — (a) High power photomicrograph of the lung of guinea-pig 1249 (figures 3 and 15) showing marked dilatation of alveoli, necrosis of capillary endothelium, desquamation and degeneration of the alveolar epithelium, and slight leukocytic iinfiltration. Hematoxylin and eosin (× 500). (b) Section of consolidated lobe of lung of guinea-pig 1448 injected with type II pneumococcus. Note the lesser dilatation of alveoli, the marked leukocytic infiltration, absence of necrosis of endothelial cells lining the alveolar capillaries, and the lesser damage to alveolar epithelium. Hematoxylin and eosin (× 500).

Fig. 20.—Section of lung of guinea-pig 947 injected intratracheally with the primary culture of the throat swab from Case 2800, which died 24 hours after injection with compressed lung from hemorrhagic fluid in the pleural cavities. Note the marked hemorrhage and edema and the poorly staining cells throughout, and the dark areas beneath the pleura and around the large blood vessels. Hematoxylin and eosin (× 50).

Plate 8

Fig. 21.—(a) Section of lung of guinea-pig 1262, injected into the trachea with the sputum of case 3175. There was a moderate amount of turbid hemorrhagic fluid in the pleural sac and a corresponding tendency of the bacteria to localize in the subpleural lymphatics as shown in the dark areas. Note the marked dilatation of the alveolar ducts and the alveoli, and the edema, hemorrhage, and desquamation of cells with relatively slight cellular infiltration throughout. Hematoxylin and eosin (× 100). (b) Diplococci and cocci in hemorrhagic, edematous areas. Gram-Weigert (× 1000).

Fig. 22.—Photomicrograph of diplococci in the edematous and hemorrhagic subpleural space of the pleura of the fetus in case 3175. Gram-Weigert (× 1000).